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(54) Title: PURINERGIC MODULATION OF SMELL

(57) Abstract: Disclosed are compositions and methods for modulating odor sensitivity, as well as screening methods for detecting compounds that modulate odor sensitivity.

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PURINERGIC MODULATION OF SMELL

I. CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. provisional application Serial No. 60/428,140, filed November 21, 2002. This application is hereby incorporated by this reference in its entirety for all of its teachings.

II. ACKNOWLEDGEMENTS

This invention was made with government support under federal grants DC04953 and DC02994 awarded by the NIH and NIDCD. The Government has certain rights to this invention.

III. BACKGROUND

A longstanding dogma, based on lack of efferent synapses, is that odor sensitivity is not modulated at the level of the olfactory receptor neurons (ORNs). The sensation of smell occurs in part by the activation of smell receptors present on the ORNs. This activation begins through contact of the chemical signature responsible for the odor with a smell receptor on the ORN. There is a need to be able to modulate sensitivity to smell, to for example, decrease sensitivity to smell in noxious environments and increase sensitivity to smell for environments in which it is desirable to smell the odors. Disclosed are methods and compositions which modulate the sensitivity to odor responsiveness.

IV. SUMMARY

As embodied and broadly described herein, the disclosed compositions and methods, in one aspect, relate to the modulation of smell. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive.

V. BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the compositions and methods and together with the description, serve to explain the principles of the compositions and methods.

Figure 1 shows the identification of purinergic receptors in the olfactory epithelium (OE). Figure 1(A) shows RT-PCR analysis of P2X₂ and P2Y₂ mRNA in rat OE and bulb. The 643-bp product represents the P2Y₂ isoform; the 499-bp product represents the P2X_{2,1} isoform, and the 292-bp product is the P2X_{2,2} isoform. Control β -actin (867 bp) and neuron specific enolase (NSE; 753 bp) RT-PCR reactions are shown. +, Indicates reverse transcribed mRNA; -, indicates omission of reverse transcriptase. Figures 1(B, C) show neonatal mouse OE showing punctate P2X₁- and P2X₄-IR (green) in olfactory marker protein (OMP)-positive (red) axons and olfactory receptor neurons (ORNs; closed arrowheads) and in OMP-negative ORNs and basal cells (open arrowheads). SC, sustentacular cell layer; BC, basal cell layer; NL, nerve layer; C, cribriform plate; NB, nerve bundle. Figure 1(D) shows neonatal mouse P2Y₂ receptor-IR (green) occurs in ORNs (closed arrowheads), in the sustentacular cell layer (open arrowheads), and in a Bowman's gland (BG, *). Figure 1(E) shows P2X₁ receptor antibody preabsorption. (LP, lamina propria) All scale bars, 20 μ M.

Figure 2 shows ATP evokes inward currents and increases intracellular Ca^{2+} in cultured mouse olfactory receptor neurons (ORNs). (A) Current responses to 10 μM ATP in two nystatin-patched ORNs held at -110 mV. Lower trace shows the ATP stimulus profile recorded separately with an open electrode. *Inset*, enlarged, compressed view of current from cell 1. (B) Confocal images from fluo-4 AM loaded ORNs taken before (left), and during (right) superfusion of 5 μM ATP. Scale bar, 50 μm . (C) Representative fluorescence (F) increases from cells a and b in (B) in response to ATP (1-10 μM). (D) Dose-response relation for maximum % $\Delta\text{F}/\text{F}$ increases, relative to 10 μM ATP (mean \pm S.E.M.; $n = 58$ ORNs for each concentration; $\text{EC}_{50} = 1.6 \mu\text{M}$). (E) Representative traces from 2 ORNs that responded to ATP (10 μM ; arrowhead) in normal Ca^{2+} and in 0 Ca^{2+} + EGTA (open bar).

Figure 3 shows that odor and purinergic receptor (P2R) agonists evoke increases in $[\text{Ca}^{2+}]_i$. See also Supplementary Information. Figures 3(A1-D4) show confocal images from a fluo-4 AM-loaded mouse olfactory epithelium (OE) slice during application of (A) odors (10 μM n-amyl acetate + 10 μM R-carvone), (B) 10 μM ATP, (C) 10 μM $\beta\gamma$ -methylene ATP ($\beta\gamma$ -MeATP), or (D) 10 μM UTP. Figures (A5-D5) show time course of odor- and P2R-agonist-evoked Ca^{2+} transients. Time points indicated by black triangles correspond to frame numbers in A1-D4. Representative odor-responsive olfactory receptor neurons (ORNs) are indicated by solid white triangles (a1-a4; 6/11 ORNs marked) and as solid lines in a5. One odor-responsive ORN (solid triangle in b1-d4) and one sustentacular cell (SC, open triangle in B1-D4) are shown in the time course (B5-D5).

Figure 4 shows a frequency of response to purinergics in ORNs and sustentacular cells. Shown are the percentages of ATP sensitive ORNs, Figure 4(A); identified by odor responsiveness; $n = 14$), and SCs, Figure 4(B); identified by location and lack of odor response; $n = 122$), that had increases in $[\text{Ca}^{2+}]_i$ evoked by non-selective purinergic receptor agonists (ATP, ATP γ S), P2Y-selective agonists (UTP, ADP, MeSADP) and P2X-selective agonists ($\beta\gamma$ -MeATP).

Figure 5 shows that ATP modulates odor responses. Figure 5(A) Suppression or Figure 5(B) enhancement of $[\text{Ca}^{2+}]_i$ due to co-application (Co-App.) compared to the summed response of ATP and odor. Shown are responses to odor (10 μM n-amyl acetate + 10 μM R-carvone), 10 μM ATP, control Ringers solution, or co-application of odor + ATP from individual mouse ORNs in olfactory epithelium slices. Figure 5(C) Bar graph showing suppression and enhancement from the 2 individual ORNs shown in A and B. The sum of the responses to individual application of ATP and odor were normalized to 1.0 (stacked bars) and the response to co-application of ATP and odor were normalized to the summed response (black bars).

Figure 6 shows the activation of specific purinergic receptor subtypes modulates odor responses. Figures 6 (A, C, E) Representative calcium transients in response to odor (10 μM n-amyl acetate + 10 μM R-carvone), 10 μM purinergic receptor (P2) agonists, or co-application of odor + P2 agonists from individual mouse ORNs in Fluo-4 AM loaded olfactory epithelium slices. Black triangles correspond to the time of loop injection of the odors or P2 agonists. Black circles correspond to the predicted peak amplitude of co-application (obtained by adding the estimated odor and P2 agonist values; refer to data analysis section for details). (B, D, F) Responses to individual application of P2 agonists and odor were normalized to the sum of

each response and averaged (stacked bars). The responses to co-application of P2 agonists and odor were normalized to the summed individual responses and averaged (black bars). The recoveries, obtained after co-application, were also normalized to the initial summed response. Bar graphs depict normalized peak Ca^{2+} transient amplitudes (mean + s.e.m.). (A-B) Co-application of 10 μM $\beta\gamma$ -methylene ATP ($\beta\gamma$ -MeATP) and odors enhanced the calcium transient amplitude in 2/16 ORNs from 2 slices. (C-D) Co-application of 10 μM $\beta\gamma$ -MeATP suppressed the calcium transient amplitude in 12/16 ORNs from 6 slices. (E-F) Co-application of 10 μM ADP βS and odors reduced the calcium transient amplitude. N = 15 ORNs from 5 slices.

Figure 7 shows examples of the growing family of ATP-gated ion channels. The predicted primary amino acid sequences of cloned P2X₁-P2X₆ receptor subtypes show that these proteins share approximately 40% sequence identity (*gray shading*) overall. Ten invariant cysteine residues (*) located within the presumptive extracellular loop may be essential for stabilizing a ligand-binding pocket through the formation of specific disulfide bonds. Putative transmembrane α -helices are delimited with black bars labeled M1 and M2. A potential pore loop region akin to that found in potassium channels corresponds to the portion of M2 denoted as (H5).

Figure 8 shows a diagram depicting a proposed transmembrane topology for P2X₂ protein showing both N- and C-terminals in the cytoplasm. Two putative membrane spanning segments (M1 and M2) traverse the lipid bilayer of the plasma membrane and are connected by a hydrophilic segment of 270 amino acids. This putative extracellular domain is shown containing two disulfide-bonded loops (S-S) and three N-linked glycosyl chains (triangles). The P2X₂ cDNA was sequenced on both strands using Sequanase. (From Brake et al., 1994).

Figure 9 shows a predicted secondary structure of the human P2Y₁-receptor. Bold circles and letters highlight amino acids that most likely contribute to the nucleotide binding site within the transmembrane regions. A change of these residues by site-directed mutagenesis caused both an increase in half-maximal concentrations of agonists such as 2-methylthio-ADP activating phospholipase C (Jiang et al. 1997) and a reduction of the antagonistic potency of the nucleotide antagonist MRS 2179 (Moro et al. 1998). The dashed lines show predicted disulphide bridges (Hoffmann et al. 1999). Glu at the position 209 and Arg at the position 287 may form additional (probably low affinity) binding sites ("meta-binding sites"; see Moro et al. 1999). Potential sites for N-linked glycosylation are not indicated (TM transmembrane region, EL extracellular loop).

Figure 10 shows the alignment of the amino acid composition of the predicted transmembrane regions (TMs) 3, 5, 6 and 7 of the human P2Y₁-, P2Y₂-, P2Y₄-, P2Y₆- and P2Y₁₁-receptors (for each subtype, the principal physiological agonist is shown *in parentheses*; please note that the human P2Y₂-receptor is activated by both UTP and ATP). **Bold letters** show a pattern of similarity in amino acid composition, which may be responsible for the pharmacological properties of the subtype. The respective residues are conserved within species. Underlined letters indicate a reduction or loss in functional responses of the (human) P2Y₁- or (murine) P2Y₂-receptor after replacement of that residue by site-directed mutagenesis. *Italic letters* indicate that a replacement had failed to change the responses (see Erb et al. 1995; Jiang et al. 1997).

Figure 11 shows the chemical structure of some key agonists and antagonists at P2 receptors. (Adapted from Windscheif, 1996).

Figure 12 shows the results of the addition of antagonists and odor stimulants on nerve cells. Representative normalized calcium transients in response to odor in the absence (A) or presence (B) of P2 receptor antagonists (100 μ M suramin 25 μ M PPADS) from individual mouse ORNs in fluo-4-AM-loaded OE slices. Filled triangles correspond to the time of loop injection of the odors. Slices were pretreated for 3 min with Ringer's solution or P2 receptor antagonists (open columns). C, Average peak calcium transient amplitudes are shown (means + SEM), as are the predicted peak amplitudes (filled circles) for the second application (n=30 ORNs from seven slices for control and n=22 ORNs from 12 slices for P2 receptor antagonists). The asterisk indicates a significant increase in $[Ca^{2+}]_i$ in the observed compared with predicted ($p < 0.024$, paired Student's t test). D, Representative traces depicting basal fluorescence levels when bath is switched at 10 sec (open column) from Ringer's solution to either P2 receptor antagonists (solid lines) or Ringer's solution (dotted lines). The fluorometric signals shown are expressed as relative fluorescence change, $\Delta F/F = (F - F_0)/F$, where F_0 is calculated from the linear rate of decay during the first 15 sec of the recording ($F_0 = mX + b$). Thus, values of 0 represent no change in fluorescence and calcium levels, negative values represent decreases in calcium, and positive values represent increases in basal calcium levels.

Figure 13 shows ATP suppresses cyclic nucleotide-induced electrical responses in olfactory epithelium. (A) shows representative EOG responses from OE slices attributable to Ringer's solution, odor, and a cyclic nucleotide mixture (100 μ M IBMX, 50 μ M CPT-cAMP, and 50 μ M 8-Br-cGMP). Filled triangles correspond to the time of loop injection of the test solutions. (B) shows representative on-cell current-clamp recording from an ORN in an OE slice. Various test solutions were superfused onto the slice for 30 seconds, indicated by the shaded region. The cell was allowed to recover for 7 minutes after each test application. Note that the coapplication of ATP (10 μ M) and the mixture suppressed the evoked membrane potential changes. (C) shows the electrical activity from each ORN was integrated from baseline, normalized to the initial cyclic nucleotide mixture response, and averaged (means + SEM). * $p < 0.05$, Newman-Keuls post hoc test. N=3 from three slices, also indicated in each column.

VI. DETAILED DESCRIPTION

The present compositions and methods can be understood more readily by reference to the following detailed description and the Examples included therein and to the Figures and their previous and following description.

Before the present compounds, compositions, articles, devices, and/or methods are disclosed and described, it is to be understood that the compositions and methods are not limited to specific synthetic methods, specific recombinant biotechnology methods unless otherwise specified, or to particular reagents unless otherwise specified, as such can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

A. Definitions

As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

5 Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other
10 endpoint, and independently of the other endpoint. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as "about" that particular value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. It is also understood that when a value is disclosed that "less than or equal to" the value, "greater than or equal to the value" and possible ranges between values are also disclosed, as appropriately understood by the skilled
15 artisan. For example, if the value "10" is disclosed then "less than or equal to 10" as well as "greater than or equal to 10" is also disclosed.

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

20 "Primers" are a subset of probes which are capable of supporting some type of enzymatic manipulation and which can hybridize with a target nucleic acid such that the enzymatic manipulation can occur. A primer can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art, which do not interfere with the enzymatic manipulation.

"Probes" are molecules capable of interacting with a target nucleic acid, typically in a sequence
25 specific manner, for example through hybridization. The hybridization of nucleic acids is well understood in the art and discussed herein. Typically a probe can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art.

"Coapplication" is defined as the application of one or more substances simultaneously, such as in the same formulation or consecutively, within a time frame such that each substance is active during a point when
30 the other substance or substances are active.

The terms "higher," "increases," "elevates," or "elevation" refer to increases above basal levels, e.g., as compared to a control. The terms "low," "lower," "reduces," or "reduction" refer to decreases below basal levels, e.g., as compared to a control. For example, basal levels are normal *in vivo* levels prior to, or in the absence of, or addition of an agent such as an agonist or antagonist.

35 The term "test compound" is defined as any compound to be tested for its ability to interact with a purinergic receptor, e.g., an epithelial Ca^{2+} entry channel agonist or antagonist. Also, "test components" include,

for example, drugs, molecules, and compounds that come from combinatorial libraries where thousands of such ligands are screened by drug class.

The terms "control levels" or "control cells" are defined as the standard by which a change is measured, for example, the controls are not subjected to the experiment, but are instead subjected to a defined set of parameters, or the controls are based on pre- or post-treatment levels.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference. The references disclosed are also individually and specifically incorporated by reference herein for the material contained in them that is discussed in the sentence in which the reference is relied upon.

Disclosed are the components to be used to prepare the disclosed compositions as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular ATP analog is disclosed and discussed and a number of modifications that can be made to a number of molecules including the ATP analog are discussed, specifically contemplated is each and every combination and permutation of the ATP analog and the modifications that are possible unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods.

It is understood that the compositions disclosed herein have certain functions, such as enhancing or reducing odor sensitivity. Disclosed herein are certain structural requirements for performing the disclosed functions, and it is understood that there are a variety of structures which can perform the same function which are related to the disclosed structures, and that these structures will ultimately achieve the same result, for example stimulation or inhibition of smell.

B. Compositions and methods

Purinergic nucleotides are important neuromodulators of auditory and visual systems. Disclosed herein is the existence and activity of purinergic receptors in mammalian olfactory epithelium, such as mouse or human, determined through immunohistochemistry, electrophysiology and calcium imaging. P2X and P2Y receptors, such as P2Y2, P2X1 and P2X4 immunoreactivity (-IR) was present on the dendrites, soma and axons of olfactory marker protein⁺ (OMP) ORNs, and in the olfactory nerve, glomerular and mitral cell layers of

the olfactory bulb. In addition, P2Y₂-IR was observed in the sustentacular cell layer of the epithelium. Application of ATP (10 μ M) onto perforated patched mouse ORNs evoked inward currents with two distinct latent periods, indicating involvement of both rapidly activating ligand-gated P2X receptors and G-protein coupled P2Y receptors, which should have a slow onset of activation. Application of ATP (10 μ M) evoked a rapid transient increase in intracellular calcium ($[Ca^{2+}]_i$). In the absence of external Ca^{2+} , ATP-evoked larger calcium transients than responses in the presence of Ca^{2+} indicating that at least part of the signal results from release from intracellular Ca^{2+} stores implicating P2Y receptor contribution to ATP-mediated Ca^{2+} transients. An olfactory epithelial (OE) slice preparation and confocal imaging was used to measure changes in $[Ca^{2+}]_i$ in fluo-4 acetoxymethyl ester (AM) loaded OE slices in response to odor and purinergic nucleotide application. Use of selective purinergic receptor agonists demonstrated that P2X and P2Y receptor agonists evoke increases in $[Ca^{2+}]_i$ in ORNs with equal frequency and that P2Y but not P2X receptor agonists evoke calcium transients in sustentacular cells. $[Ca^{2+}]_i$ levels were measured in response to odor, ATP, or odor + ATP. In most cells, ATP reduced the summed odor-induced changes in Ca^{2+} however, some cells exhibited an increase in evoked $[Ca^{2+}]_i$ increase, indicating an increased effect. Collectively, the data indicates that P2X and P2Y receptor subtypes are expressed in the olfactory epithelium and that P2X and P2Y agonists and antagonists modulation of odor responses, such as the agonist ATP, can be dependent on the subtype(s) of purinergic receptors expressed.

Disclosed herein is direct evidence that ATP and ATP analogs modulate odor responses in olfactory receptor neurons. ATP released in the olfactory epithelium following noxious stimuli provides a physiological source for a neuromodulatory substance independent of efferent innervation. Peripheral ATP-mediated odor suppression is a mechanism for reduced olfactory sensitivity during exposure to olfactotoxins. Methods for modulating the sensitivity to smell of a subject are disclosed.

1. P2X and P2Y purinergic receptors

P2X receptors form Ca^{2+} -permeable nonselective cation channels that allow Ca^{2+} influx from the extracellular fluid. Most of the 8 functional P2Y receptors identified to date act via G-protein coupling to activate phospholipase C, leading to production of inositol triphosphates and mobilization of Ca^{2+} from internal stores (Dubyak and el-Moatassim, 1993); however, a few P2Y receptors couple to adenylate cyclase (Ralevic and Burnstock, 1998). All of the components of both transduction pathways have been identified in ORNs (Schild and Restrepo, 1998).

Although purines are odorants for aquatic vertebrates (Kang and Caprio, 1995) and invertebrates (Carr, W. E., et al., Environ. Health Perspect. 71, 31-46 (1987)), disclosed herein, extracellular purinergic nucleotides and their receptors in mammalian, such as human, olfactory epithelium exist. Disclosed herein, RT-PCR and immunohistochemistry and physiological studies, show that sustentacular support cells express P2Y receptors and that ORNs express both P2X and P2Y receptors. Regionally localized purinergic receptors are consistent with extracellular ATP having multiple roles in the peripheral olfactory system. Furthermore, it is shown herein that ATP differentially modulates the odor responsiveness of ORNs. This indicates that the complement of P2X and/or P2Y receptor subtypes expressed in the ORN can determine whether the odor response is enhanced or inhibited in the presence of ATP.

There are two main families of purine receptors, adenosine or P1 receptors, and P2 receptors, recognizing primarily ATP, ADP, UTP, and UDP (Table 1). Adenosine/P1 receptors couple to G proteins and have been further subdivided, based on molecular, biochemical, and pharmacological evidence into four subtypes, A₁, A_{2A}, A_{2B}, and A₃. In contrast, P2 receptors divide into two families of ligand-gated ion channels and G protein-coupled receptors termed P2X and P2Y receptors, respectively. For example, Table 1 sets forth seven mammalian P2X receptors (P2X₁₋₇) and five mammalian P2Y receptors (P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y₁₁) which have been cloned and characterized.

TABLE 1: Families of receptors for purines and pyrimidines

(Modified from Ralevic V, Burnstock G. Pharmacol Rev 1998 Sep;50(3):413-92.)

	Adenosine/P1 receptors	P2 receptors	
Natural ligands	Adenosine	ATP, ADP, UTP, UDP, Adenine dinucleotides	
Subgroup	—	P2X	P2Y
Type	G protein-coupled	Ion channel Nonselective pore	G protein-coupled
Subtypes	A ₁ , A _{2A} , A _{2B} , A ₃	P2X ₁₋₇ , P2X _n	P2Y ₁ , P2Y ₂ , P2Y ₄ , P2Y ₆ , P2Y ₁₁ , P2Y _{ADP} (or P2 _T) Uridine nucleotide-specific

P2X receptors are ATP-gated ion channels which mediate rapid (within 10 ms) and selective permeability to cations (Na⁺, K⁺ and Ca²⁺) (Bean, 1992; Dubyak and el-Moatassim, 1993; North, 1996). They are typically found on excitable cells (smooth muscle cells, neurons, and glial cells) and mediate fast excitatory neurotransmission to ATP in both the central and peripheral nervous systems. This contrasts with the slower response (less than 100 ms) to ATP acting at metabotropic P2Y receptors, which involves coupling to G proteins and second-messenger systems. Seven functional P2X receptor proteins (P2X₁ to P2X₇) have been cloned and form homomeric ion channels with distinct pharmacological profiles when expressed in *Xenopus* oocytes (Table 2). The P2X₇ receptor is considered separately below, because it is functionally unique among P2X receptors in being able to act as a non-selective pore.

Functional cDNAs encoding the first two members of this family, P2X₁ and P2X₂, were isolated from vas deferens smooth muscle and PC12 pheochromocytoma cells, respectively, using an expression cloning strategy in *Xenopus* oocytes (Brake et al 1994, Valera et al 1994). In each case, expression of a single cDNA clone in oocytes or transfected mammalian cells is sufficient to direct the synthesis of functional, presumably homomeric ATP-gated ion channel complexes on the surface of these cells. P2X₁ and P2X₂ receptors are clearly related at the level of primary amino acid sequence and predicted secondary structure. Four additional members of this channel family have now been cloned using PCR-based screening strategies (Bo et al 1995, Chen et al 1995, Lewis et al 1995, Buell et al 1996, Collo et al 1996, Seguela et al 1996). All six subtypes share approximately 40% sequence identity distributed fairly evenly over their length, which ranges from 379 to 472 residues (Figure 7).

TABLE 2 Cloned P2X receptors and typical activity profiles

Receptor	Accession number	cDNA library source	Agonist activity	References
P2X₁ (399 amino acids (aa))	X83688	Human urinary bladder	ATP > α,β -meATP	Evans <i>et al.</i> , 1994; Valera <i>et al.</i> , 1995; Longhurst <i>et al.</i> , 1996
	X80477	Rat vas deferens	2MeSATP > ATP > α,β -meATP	Valera <i>et al.</i> , 1994
	X84896	Mouse urinary bladder		Valera <i>et al.</i> , 1996
P2X₂ (472 aa)	U14414	Rat PC12 cells	2MeSATP > ATP; α,β -meATP inactive	Brake <i>et al.</i> , 1994
P2X_{2(b)}^a (401 aa)	Y09910	Rat cerebellum	2MeSATP = ATP = α,β -meATP	Brändle <i>et al.</i> , 1997; Simon <i>et al.</i> , 1997
P2X₃ (397 aa)	Y07684	Human heart, spinal cord	2MeSATP > ATP > α,β -meATP	Garcia-Guzman <i>et al.</i> , 1997b
	X90651	Rat dorsal root ganglion cells	2MeSATP > ATP > α,β -meATP > UTP	Chen <i>et al.</i> , 1995a
	X91167	Rat dorsal root ganglion cells	ATP > 2MeSATP > α,β -meATP	Lewis <i>et al.</i> , 1995
P2X₄ (388 aa)	Y07684	Human brain	ATP » 2MeSATP ≥ CTP > α,β -meATP	Garcia-Guzman <i>et al.</i> , 1997a
	X93565	Rat brain	ATP » 2MeSATP ≥ CTP > α,β -meATP	Soto <i>et al.</i> , 1996a
	U32497	Rat brain	ATP > 2MeSATP » α,β -meATP	Séguéla <i>et al.</i> , 1996
	X91200	Rat hippocampus	ATP > 2MeSATP » α,β -meATP	Bo <i>et al.</i> , 1995
	X87763	Rat superior cervical ganglion	ATP; α,β -meATP inactive	Buell <i>et al.</i> , 1996b
	U47031	Rat pancreatic islet	ATP, ADP, 2MeSATP » α,β -meATP	Wang <i>et al.</i> , 1996
P2X₅ (417 aa) (455 aa)	X92069	Rat ganglia	ATP > 2MeSATP > ADP α,β -meATP inactive	Collo <i>et al.</i> , 1996
	X97328	Rat heart	ATP > 2MeSATP > ADP	Garcia-Guzman <i>et al.</i> , 1996
P2X₆ (379 aa)	X92070	Rat superior cervical ganglion	ATP > 2MeSATP > ADP; α,β -meATP inactive	Collo <i>et al.</i> , 1996
	X97376	Rat brain		Soto <i>et al.</i> , 1996b
P2X₇ (595 aa)		Mouse macrophage	BzATP > ATP > UTP ATP > UTP > BzATP	Nuttie <i>et al.</i> , 1993
	X95882	Rat macrophage and brain	BzATP > ATP > 2MeSATP > ADP; UTP inactive	Surprenant <i>et al.</i> , 1996
		Human monocytes	BzATP > ATP	Rassendren <i>et al.</i> , 1997

^a Splice variant, also termed P2X₂₋₂.All references are herein incorporated by reference at least for material related to a P2X or P2Y receptor.
Modified from Ralevic V, Burnstock G. Pharmacol Rev 1998 Sep;50(3):413-92.

Based on the amino acid sequences of cloned P2X receptor subunits, structural features of P2X receptors have been predicted. The P2X proteins that have been cloned are receptor subunits, not actual receptors since a single 2 transmembrane subunit alone cannot form an ion channel. The proteins have 379 to 472 amino acids and are believed to insert into the cell membrane to form a pore comprising two hydrophobic transmembrane domains (M1 and M2), with much of the protein occurring extracellularly as an intervening hydrophilic loop (figure 8). It is presumed that both amino- and carboxyl-termini are located on the intracellular side of the membrane. Based on genetic studies in *C. elegans*, the M2 domain of these channels forms an amphipathic α -helix whose hydrophilic face lines the pore (Hong & Driscoll 1994). Interestingly, helical wheel plots of M2 domains from each of the cloned P2X subunits show that they have similar potential to form amphipathic α -helices, despite the limited sequence homology in this region. In addition, some P2X subunits contain a region, that resembles the H5 pore loop domain of potassium channels, and it is possible that this segment (just amino-terminal to M2), also contributes to the pore of ATP-gated channels. However, there is considerable variability in the H5 domain consensus sequence, and its location relative to M2, among the six cloned P2X receptor subtypes. The overall structure of the receptor most closely resembles that of amiloride-sensitive epithelial Na^+ channels. The putative extracellular loop of cloned receptors P2X₁ to P2X₇ has 10 conserved cysteine residues, 14 conserved glycine residues and 2 to 6 potential N-linked glycosylation sites. It is believed that disulfide bridges may form the structural constraints needed to couple the ATP-binding site to the ion pore. Most of the conserved regions are in the extracellular loop, with the transmembrane domains being less well-conserved. As for other ligand-gated receptors, P2X receptors are believed to form a heterologous complex in biological tissues. Although their subunit stoichiometry is unknown, SDS polyacrylamide gel electrophoresis estimates of the relative molecular mass of the recombinant P2X₁ and P2X₃ receptors determined under non-denaturing conditions (Nicke *et al.*, 1998) suggest a combination of three subunits (or multiples of three subunits).

Both cloned P2X₇ and endogenous P2X₇-like receptors are unique in that, under physiological conditions they are selectively permeable to small cations only, but in the presence of low divalent cation levels and ATP, the P2X₇ channel can convert to a pore, permeable to small molecules as well as ions. The P2X₇ receptor and its endogenous counterpart is structurally similar to other P2X receptors, except for the fact that it has a significantly longer intracellular C-terminal (240 amino acids) than other P2X receptors, of which at least the last 177 amino acids are crucial for the induction of the non-selective pore (Surprenant *et al.*, 1996). Brief activation of the recombinant P2X₇ receptor and its endogenous counterpart causes rapid membrane depolarization and cation influx and is a reversible process. However, sustained activation causes an increase in permeability by allowing bidirectional transport of a variety of ions including Na^+ , K^+ , and Ca^{2+} and small molecules with a molecular weight of less than or equal to 900 daltons, except in lymphocytes where the limit is 200-300 daltons. This effect is associated with cytotoxicity. Although cation function of the receptor is retained in a truncated P2X₇ receptor lacking the last 177 residues, the increased permeability is lost suggesting involvement of the cytoplasmic C terminus. The disclosed results indicate that the P2X₇ receptor is not typically present in mammalian olfactory epithelium.

P2Y receptors are purine and pyrimidine nucleotide receptors that are coupled to G proteins. Most P2Y receptors act via G protein coupling to activate PLC leading to the formation of IP₃ and mobilization of intracellular Ca²⁺. Coupling to adenylate cyclase by some P2Y receptors has also been described. The response time of P2Y receptors is longer than that of the rapid responses mediated by P2X receptors because it involves second-messenger systems and/or ionic conductances mediated by G protein coupling. Five mammalian P2Y
5 receptors (P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y₁₁) have been cloned, and functionally characterized and show distinct pharmacological profiles (Table 3).

TABLE 3: Cloned P2Y receptors

Receptor	Accession number	cDNA library source	Agonist activity	References
P2Y ₁ (362 amino acids (aa))	S81950	Human brain Human prostate and ovary	2MeSATP > ATP » UTP 2MeSATP > ATP = ADP	Schachter <i>et al.</i> , 1996 Janssens <i>et al.</i> , 1996
	Z49205	Human placenta		Léon <i>et al.</i> , 1995, 1997
	U42030	Human HEL cells		Ayyanathan <i>et al.</i> , 1996
	X87628	Bovine endothelium	2MeSATP = ADP > ATP » UTP	Henderson <i>et al.</i> , 1995
	U22830	Rat insulinoma cells	2MeSATP > 2Cl-ATP > ATP (α , β -meATP inactive)	Tokuyama <i>et al.</i> , 1995
		Rat ileal myocytes	2MeSATP = 2ClATP > ADP > ATP (UTP inactive)	Pacaud <i>et al.</i> , 1996
	U22829	Mouse insulinoma cells		Tokuyama <i>et al.</i> , 1995
	U09842	Turkey brain	2MeSATP > ADP > ATP; (UTP inactive)	Filtz <i>et al.</i> , 1994
	X73268	Chick brain	2MeSATP > ATP > ADP; (UTP inactive)	Webb <i>et al.</i> , 1993b
P2Y ₂ (373 aa)	U07225	Human CF/T43 epithelial cells Human bone Rat microvascular coronary EC	ATP = UTP » 2MeSATP	Parr <i>et al.</i> , 1995 Bowler <i>et al.</i> , 1995 Gödecke <i>et al.</i> , 1996
	U09402	Rat alveolar type II cells	ATP = UTP	Rice <i>et al.</i> , 1995
	L46865	Rat pituitary	ATP = UTP > ADP = UDP > GTP	Chen <i>et al.</i> , 1996b
	U56839	Wistar Kyoto rat ^a		Seye <i>et al.</i> , 1996
	NM_008773	Mouse NG108-15 neuroblastoma cells	ATP = UTP > ATP _γ S » 2MeSATP	Lustig <i>et al.</i> , 1993
p2y3 ^b (328 aa)	X98283	Chick brain	UDP > UTP > ADP > 2MeSATP > ATP	Webb <i>et al.</i> , 1995, 1996a
P2Y ₄ (352 aa)	X91852	Human placenta Human placenta	UTP > ATP = ADP ^c	Communi <i>et al.</i> , 1996b Stam <i>et al.</i> , 1996
	U40223	Human chromosome X	UTP > UDP (ATP inactive)	Nguyen <i>et al.</i> , 1996
	Y14705	Rat heart	ATP = UTP = ADP = ITP = ATP _γ S = 2MeSATP = Ap ₄ A > UDP	Bogdanov <i>et al.</i> , 1998
P2Y ₆ (379 aa)	X97058	Human placenta and spleen	UDP > UTP > ADP > 2MeSATP » ATP	Communi <i>et al.</i> , 1996b
	NM_057124	Rat aortic smooth muscle	UTP > ADP = 2MeSATP > ATP	Chang <i>et al.</i> , 1995
	U52464	Activated T-cells		Southey <i>et al.</i> , 1996
P2Y ₁₁ (371 aa)	371	Human placenta	ATP > 2MeSATP >>> ADP; (UTP, UDP inactive)	Communi <i>et al.</i> , 1997

^a Tissue not specified.^b p2y3 may be the chick homologue of the mammalian P2Y₆ receptor.^c The reported activity of UDP at the P2Y₄ receptor has been shown to be caused by UTP present as a contaminant. Each of the references herein is incorporated by reference at least for material related to P2Y receptors

Modified from Ralevic V, Burnstock G. Pharmacol Rev 1998 Sep;50(3):413-92.

P2Y receptors are 308 to 377 amino acid proteins with a mass of 41 to 53 kDa after glycosylation. The tertiary structure of P2Y receptors is similar to that of other seven transmembrane domain G protein-coupled receptors (Figure 9). A model of the P2Y receptor, based on the primary sequence of the P2Y₁ receptor and using the structural homolog rhodopsin as a G protein-coupled receptor template, has identified positively charged amino acid residues in transmembrane regions 3, 6, and 7 that may be involved in ligand binding by electrostatic interactions with the phosphates of ATP (Van Rhee *et al.*, 1995) (Figure 10). Several of these amino acids are conserved in other G protein-coupled receptors. Site-directed mutagenesis of the P2Y₂ receptor to convert positively charged amino acids in transmembrane regions 6 and 7 to neutral amino acids causes a 100- to 850-fold decrease in the potency of ATP and UTP, which suggests a role for these amino acids in binding purines and pyrimidines (Erb *et al.*, 1995). In contrast, in the human P2Y₁ receptor, the most important residues for ATP binding are in transmembrane regions 3 and 7 on the exofacial side of the receptor (Jiang *et al.*, 1997).

2. ATP and ATP analog activity on purinergic receptors

Extracellular ATP plays an important role in cellular signaling and acts as a cotransmitter or neuromodulator in sensory systems (Thorne and Housley, 1996). In the olfactory system, ATP can be released from synaptic vesicles in trigeminal afferents that innervate the olfactory epithelium and detect noxious chemicals (Finger *et al.*, 1990; Getchell and Getchell, 1992), or via plasma membrane nucleotide transport proteins (Roman *et al.*, 1997). Furthermore, ischemic, stressed, and injured cells release ATP in large amounts. A recent toxicology study (Kilgour *et al.*, 2000) showed that when the olfactory epithelium was damaged by noxious fumes [ATP]_i significantly decreased, whereas stimulation that did not damage the olfactory epithelium did not affect [ATP]_i. In addition to toxic chemicals, prolonged exposure to concentrated odors, such as peppermint, will damage olfactory receptor neurons (ORNs) and induce expression of stress indicators (heat shock proteins) in sustentacular support cells (Carr *et al.*, 2001). Therefore, both trigeminal and odorous stimulation provide sources for extracellular ATP in olfactory epithelium.

Once released, ATP can have autocrine or paracrine effects. Very low concentrations of ATP activate the two subtypes (P2X and P2Y) of purinergic receptors (0.1-10 μ M) (Ralevic and Burnstock, 1998; Schwiebert and Kishore, 2001). Through either of these receptor subtypes, ATP is able to stimulate an increase in [Ca²⁺]_i (Illes *et al.*, 2000; Koshimizu *et al.*, 2000; Ralevic and Burnstock, 1998).

P2 receptors have broad natural ligand specificity, recognizing ATP, ADP, UTP, UDP, and the diadenosine polyphosphates (Table 1). The chemical structures of some principal P2 receptor agonists and antagonists are illustrated in Figure 11. For example, P2X selective agonists are the stable ATP analogs α,β -meATP and β,γ -meATP, which if effective, strongly imply actions at P2X receptors (typically at P2X₁ and P2X₃ subtypes) and are generally inactive at P2Y receptors. Also useful are ADP, adenosine 5'-O-(2-thiodiphosphate)(ADP β S,) and UTP, as these are agonists at some P2Y receptors, but are weak or inactive at P2X receptors.

TABLE 4 Exemplary P2 receptor signal transduction mechanisms, agonists, and antagonists

Family	P2X	P2Y
Receptor type	Ion channel: Nonselective pore ^a	G protein-coupled: G _{q/11} , G _i ^b
Signaling pathway	Not applicable	PLC, AC ^c K ⁺ channels ^d , PLC _{PC} ^e , PLA ₂ ^f , PLD ^f , PKC, MAPK ^g
Effectors	Ca ²⁺ » Na ⁺ > K ⁺	↑IP ₃ , ↑Ca ²⁺ , ↑DAG ↓cAMP ^e , Ca ²⁺ , Cl ⁻ , K ⁺ currents ^h
Nonselective Agonists	ATP ⁱ , ATPγS, 2MeSATP, Ap ₄ A ^j	ATP ⁱ , ATPγS, 2MeSATP, Ap ₄ A ^j
P2X/P2Y-selective Agonists	α,β-meATP ⁱ , β,γ-meATP ⁱ , BzATP ^a	ADP ^c , UTP ^m , UTPγS ^j , UDP ⁿ , 2Cl-ADP ^c , 2MeSADP ^c , ADPβS ^c , ADPβF ^c
Nonselective Antagonists	Suramin, PPADS, Iso-PPADS, P5P, Reactive blue 2, Reactive Red, Trypan Blue, Evans Blue, DIDS	Suramin, PPADS, Iso-PPADS, P5P, Reactive blue 2, Reactive Red, Trypan Blue, Evans Blue, DIDS
P2X/P2Y-selective Antagonists	NF023, NF279, KN-62 ^a	ARL 67085 ^o , FPL 66096 ^o , A3P5PS ^k , MRS 2179 ^k , 2-hexylthio-ATP ^p , 2-cyclohexylthio-ATP ^p

^a P2X₇ and endogenous P2X₇-like receptor.

^b P2Y₁ and endogenous P2Y₁-like receptors acting through PLC couple to G_{q/11} proteins; P2Y₁ and endogenous P2Y₁-like receptors acting through adenylate cyclase couple to G_i proteins; P2Y₂ and endogenous P2Y₂-like receptors, P2Y₄ and P2Y_{ADP} receptors couple to G_{q/11} and G_i proteins; p2y3 and P2Y₆ receptors couple to G_{q/11} proteins.

^c P2Y₁ and endogenous P2Y₁-like receptors and P2Y_{ADP} receptors.

^d Some endogenous P2Y₁-like receptors activate K⁺ channels via interactions with their G protein subunits.

^e P2Y₁ and endogenous P2Y₁-like receptor signaling; possibly downstream of PKC.

^f P2Y₁ and P2Y₂ receptors and their endogenous counterparts; signaling possibly downstream of PKC.

^g P2Y₁ and P2Y₂ receptors and their endogenous counterparts; signaling downstream of PKC.

^h Secondary to activation of PLC, although activation of K⁺ currents by some endogenous P2Y₁-like receptors is via direct interactions with G protein subunits.

ⁱ P2Y₁ and P2Y₂ receptors and their endogenous counterparts; ATP is an antagonist at P2Y_{ADP} receptors.

^j P2Y₂ and endogenous P2Y₂-like receptors.

^k P2Y₁ and endogenous P2Y₁-like receptors.

^l P2X₁, P2X₃ and heteromeric P2X₂P2X₃ receptors.

^m P2Y₂ and endogenous P2Y₂-like receptors and P2Y₄ receptors.

ⁿ P2Y₆ receptor. ^o P2Y_{ADP}. ^p P2Y₁ and endogenous P2Y₁-like receptors coupled to AC.

Abbreviations: AC, adenylate cyclase; ADPβF, adenosine 5'-O-(2-fluoro)-diphosphate; ADPβS, adenosine 5'-O-(2-thio)-diphosphate; cAMP, adenosine 3',5'-cyclic monophosphate; A3P5PS, adenosine 3'-phosphate 5'-phosphosulfate; ARL 67085, 6-N,N-diethyl-D-β,γ-dibromomethylene ATP; ATPγS, adenosine 5'-O-(3-thiotriphosphate); BzATP, 3'-O-(4-benzoyl)benzoyl ATP; DAG, diacylglycerol; DIDS, 4,4'-diisothiocyanatostilbene-2,2'-disulfonate; FPL 66096, 2-propylthio-D-β,γ-difluoromethylene ATP; IP₃, inositol 1,4,5-trisphosphate; KN-62, 1-[N,O-bis(5-isoquinolinesulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine; Iso-PPADS, pyridoxal phosphate-6-azophenyl-2',5'-disulfonic acid; MAPK, mitogen-activated protein kinase; α,β-meATP, α,β-methylene ATP; β,γ-meATP, β,γ-methylene ATP; 2MeSADP, 2-methylthio ADP; 2MeSATP, 2-methylthio ATP; MRS 2179, N⁶-methyl modification of 2'-deoxyadenosine 3',5'-bisphosphate; NF023, symmetrical 3'-urea of 8-(benzamido)naphthalene-1,3,5-trisulfonic acid; NF279, 8,8'-(carbonylbis(imino-4,1-phenylenecarbonylimino))bis(1,3,5-naphthalenetrisulfonic acid); P5P, pyridoxal-5-phosphate; PLC_{PC}, phosphatidylcholine-specific phospholipase C; PKC, protein kinase C; PLA₂, phospholipase A₂; PLC, phospholipase C; PLD, phospholipase D; PPADS, pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid; suramin, 8-(3-benzamido-4-methylbenzamido)-naphthalene-1,3,5-trisulfonic acid; UTPγS, uridine 5'-O-(3-thiotriphosphate).

Modified from Ralevic V, Burnstock G. Pharmacol Rev 1998 Sep;50(3):413-92.

3. Inhibiting olfactory response

As discussed herein if in an odor-ATP or analog assay, the calcium transient evoked by co-application is less than the sum of the calcium transients evoked by the individual components then there is an inhibiting

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effect on the olfactory response. Of the cells that responded to odor, 62% (21/26 cells) exhibited a significant decrease in the summed $[Ca^{2+}]_i$ increase. The mean suppression of all cells was $57\% \pm 5\%$ (paired t-test, $p = 0.01$, $n = 26$). Thus, ATP reduced the expected combined effect of the ATP and the odor, and thus will act as an odor suppressant. Typically, activation of P2Y receptors reduced sensitivity to odors. For example, the P2Y selective agonists UTP and ADP- β S suppressed the co-application evoked calcium transient indicating they can act as odor suppressants. As discussed in the Examples, similar experiments were performed with P2X and P2Y selective agonists giving similar results.

Disclosed are compositions and methods for inhibiting the odor response of an ORN. Inhibition of the response can be determined by performing the transient calcium flux assays as discussed herein. Typically these assays can be performed in the presence or absence of the odor. Thus, compositions which inhibit the ORN response can be compositions which in a calcium transient flux assay, the presence of the composition and the odor together, produces a transient calcium flux that is less than the sum of the odor induced flux alone and the composition induced flux alone. For example, if the amount of calcium flux in the presence of a composition and an odor is A, and the amount of the calcium flux in the presence of the composition alone is B and the amount of the calcium flux in the presence of the odor alone is C then if $A < B + C$, the composition can be said to inhibit the ORN response and the composition can inhibit a smell response. Disclosed are compositions wherein the combined effect of the composition and odor (A) is less than or equal to 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, or 1% of the summed effect (B+C). It is understood that these numbers can be averages, with variances, and that these types of statistics can be employed, as discussed herein, to determine if the combined effect is less than the summed effect.

It is also understood that when the combined effect A, is less than the summed effect (B+C) that this can be expressed as a ratio of $A/(B+C)$ and that ratios less than 1 indicate compositions that inhibit the ORN effect. For example, disclosed are compositions that have a ratio of less than or equal to 0.01, 0.03, 0.05, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.52, 0.55, 0.6, 0.64, 0.65, 0.69, 0.7, 0.72, 0.75, 0.80, 0.83, 0.85, 0.87, 0.90, 0.92, 0.95, 0.97, or 0.99. The ratio can be expressed in terms of a range of these individual ratios, such as 0.72 to 0.92, for example, or 0.52 to 0.64, or 0.69 to 0.83.

Thus, when an ORN is expressing the P2Y receptor and a P2Y selective agonist or a non-selective purinergic agonist is applied, odor response is suppressed. Likewise, when an ORN is expressing a P2X receptor and a P2X selective agonist is applied, the odor response is typically suppressed. Also, when both P2X and P2Y receptors are present on an ORN, and either a P2Y selective agonist, a P2X selective agonist, or a nonselective agonist is applied, the odor response is suppressed. Combinations of selective and non-selective agonists can be applied, and P2X and P2Y receptors can be suppressed depending on the combination of agonists in the mixture.

Disclosed are P2X selective agonists and P2Y selective agonists. Disclosed are P2X directed agonists and P2Y directed agonists. In certain embodiments, a P2X directed agonist is any agonist that has a greater effect on a P2X receptor than on a P2Y receptor. Likewise, in certain embodiments, a P2Y directed agonist is any agonist that has a greater effect on a P2Y receptor than on a P2X receptor. In other embodiments, P2X

agonists and P2Y agonists can be determined by comparing the activity to known selective agonists, such as those discussed herein. It is understood that the level of activity of each selective agonist discussed herein, is disclosed. Also disclosed are P2 agonists that interact with any P2 receptor. It is understood that many P2X and P2Y agonists can be both a selective agonist as well as a directed agonist. For example, UTP can be a selective and a directed P2Y agonist.

Just as P2X and P2Y agonists inhibit ORN response to odor stimulants, so too, antagonists of P2X and P2Y receptors can lead to an enhancement of the smell response. P2X antagonists, such as those disclosed in Table 4, for example, act at P2X receptors and P2Y antagonists, such as those disclosed in Table 4, for example, act at P2Y receptors, and thus can be stimulators of odor responsiveness. It is understood that the assays, measurements, and functional limitations, as discussed, herein for agonists are applicable for antagonists as well. Thus, for example, antagonists can be assayed in a calcium flux assay, but an antagonist would be considered a composition (B) that does not evoke a response in the calcium flux assay alone, i.e., $B=0$. However, when a composition (B) is co-applied with an odor (C), the combined odor and composition effect (A), would be greater than the effect of odor alone (C), or composition alone (B) and thus, $A > (B+C)$ or since $B=0$, $A > C$.

Typically, antagonists have an opposite effect on a receptor than an agonist, and application of the disclosed methods and limitations can be thus applied to antagonists, as they were for agonists.

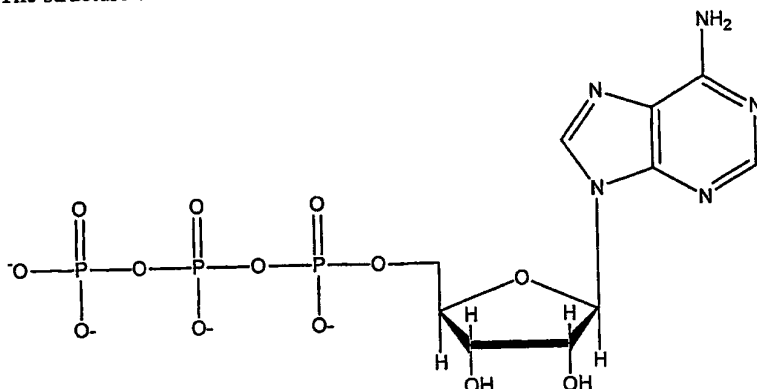
Disclosed are P2X selective antagonists and P2Y selective antagonists. Disclosed are P2X directed antagonists and P2Y directed antagonists. In certain embodiments, a P2X directed antagonist is any antagonist that has a greater effect on a P2X receptor than on a P2Y receptor. Likewise, in certain embodiments, a P2Y directed antagonist is any antagonist that has a greater effect on a P2Y receptor than on a P2X receptor. In other embodiments, P2X antagonist and P2Y antagonist can be determined by comparing the activity to known selective antagonists, such as those discussed herein. It is understood that the level of activity of each selective antagonist discussed herein, is disclosed. Also disclosed are P2 antagonists that interact with any P2 receptor. It is understood that many P2X and P2Y antagonists can be both a selective antagonist as well as a directed antagonists.

Disclosed herein are methods of modulating odor sensitivity in a subject, comprising administering a composition to the subject, wherein the composition is an antagonist of a P2X or P2Y purinergic receptor. The antagonist can increase the odor sensitivity of the subject, which can be desirable to those with olfactory impairments. Increasing odor sensitivity is also desirable in conjunction with a pleasant smell. The antagonist can reduce basal Ca^{2+} levels in olfactory receptor neurons which will make the neurons more excitable during subsequent odor stimulation thereby increasing the odor sensitivity of the subject. The antagonist can increase the ratio of observed coapplication-evoked calcium transient compared to the individual odor peak amplitudes in a cell activation assay, as discussed above.

C. Compositions

1. ATP and ATP analogs

The structure of ATP is shown in Formula 1.



5 Formula 1

There are many analogs of ATP that can be made. For example, analogs can be made at the base moiety, the sugar moiety, and the phosphate moiety, as discussed herein. The base moiety can be considered as adenin-9-yl (A). Many modifications can take place at this moiety. The sugar moiety of a nucleotide is typically a ribose or a deoxyribose. The phosphate moiety of a nucleotide is typically pentavalent phosphate.

10 A non-limiting example of a nucleotide would be 3'-AMP (3'-adenosine monophosphate), ADP, and ATP.

ATP analogs can have modifications to the base moiety which would include natural and synthetic modifications of A, such as hypoxanthin-9-yl (I), and 2-aminoadenin-9-yl, 2-aminoadenine, xanthine, 6-methyl and other alkyl derivatives of adenine, 2-propyl and other alkyl derivatives of adenine, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines, 7-methyladenine, 8-azaadenine,

15 7-deazaadenine and 3-deazaadenine, and O-6 substituted adenines, including 2-aminopropyladenine.

ATP analogs can also include modifications of the sugar moiety. Modifications to the sugar moiety would include natural modifications of the ribose and deoxy ribose as well as synthetic modifications. Sugar modifications include but are not limited to the following modifications at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl

20 can be substituted or unsubstituted C₁ to C₁₀, alkyl or C₂ to C₁₀ alkenyl and alkynyl. 2' sugar modifications also include but are not limited to -O[(CH₂)_n O]_m CH₃, -O(CH₂)_n OCH₃, -O(CH₂)_n NH₂, -O(CH₂)_n CH₃, -O(CH₂)_n -ONH₂, and -O(CH₂)_n ON[(CH₂)_n CH₃]₂, where n and m are from 1 to about 10.

ATP analogs can have other modifications at the 2' position and include but are not limited to: C₁ to C₁₀ lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂ CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, and polyalkylamino. Modified sugars would also include those that contain modifications at the bridging ring oxygen, such as CH₂ and S. Nucleotide sugar analogs can also have sugar mimetics such as cyclobutyl

25 moieties in place of the pentofuranosyl sugar. There are numerous United States patents that teach the preparation of such modified sugar structures such as 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300;

30

5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, each of which is herein incorporated by reference in its entirety.

ATP analogs can also be modified at the phosphate moiety. Modified phosphate moieties include but are not limited to those that can be modified so that the linkage between two nucleotides contains a phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl and other alkyl phosphonates including 3'-alkylene phosphonate and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates.

It is understood that nucleotide analogs need only contain a single modification, but can also contain multiple modifications within one of the moieties or between different moieties.

Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

Nucleotide substitutes are nucleotides or nucleotide analogs that have had the phosphate moiety and/or sugar moieties replaced. Nucleotide substitutes do not contain a standard phosphorus atom. Substitutes for the phosphate can be for example, short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts. Numerous United States patents disclose how to make and use these types of phosphate replacements and include but are not limited to 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

Disclosed are uses for non-selective, P2X selective and P2Y selective ATP analogs. Furthermore, there are ATP selective agonists and ATP selective antagonists. For example, non-selective purinergic receptor agonists are ATP, ATPyS, and AMP (Table 4). For example, P2Y-'selective' agonists are UTP, ADP, and MeS-ADP (Table 4). In addition, an example of a P2X-'selective' agonist is β -methylene ATP (Table 4). Suramin and pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) are examples of non-specific antagonists.

2. General composition information

a) Sequence similarities

It is understood that as discussed herein the use of the terms homology and identity mean the same thing as similarity. Thus, for example, if the use of the word homology is used between two non-natural sequences it is understood that this is not necessarily indicating an evolutionary relationship between these two sequences, but rather is looking at the similarity or relatedness between their nucleic acid sequences. Many of the methods for determining homology between two evolutionarily related molecules are routinely applied to any two or more nucleic acids or proteins for the purpose of measuring sequence similarity regardless of whether they are evolutionarily related or not.

For example, SEQ ID NO:1 represents a version of a P2X receptor. All fragments of the P2X receptor, as well as the other proteins, such as receptors discussed herein, are considered disclosed.

In general, it is understood that one way to define any known variants and derivatives or those that might arise, of the disclosed genes and proteins herein, is through defining the variants and derivatives in terms of homology to specific known sequences. This identity of particular sequences disclosed herein is also discussed elsewhere herein. In general, variants of genes and proteins herein disclosed typically have at least, about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent homology to the stated sequence or the native sequence. Those of skill in the art readily understand how to determine the homology of two proteins or nucleic acids, such as genes. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, (1989) which are herein incorporated by reference for at least material related to nucleic acid alignment. It is understood that any of the methods typically can be used and that in certain instances the results of these various methods can differ, but the skilled artisan understands if identity is found with at least one of these methods, the sequences would be said to have the stated identity, and be disclosed herein.

For example, as used herein, a sequence recited as having a particular percent homology to another sequence refers to sequences that have the recited homology as calculated by any one or more of the calculation methods described above. For example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second

sequence using the Zuker calculation method even if the first sequence does not have 80 percent homology to the second sequence as calculated by any of the other calculation methods. As another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using both the Zuker calculation method and the Pearson and Lipman calculation method even if the first sequence does not have 80 percent homology to the second sequence as calculated by the Smith and Waterman calculation method, the Needleman and Wunsch calculation method, the Jaeger calculation methods, or any of the other calculation methods. As yet another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using each of calculation methods (although, in practice, the different calculation methods will often result in different calculated homology percentages).

b) Hybridization

The term hybridization typically means a sequence driven interaction between at least two nucleic acid molecules, such as a primer or a probe and a gene. Sequence driven interaction means an interaction that occurs between two nucleotides or nucleotide analogs or nucleotide derivatives in a nucleotide specific manner. For example, G interacting with C or A interacting with T are sequence driven interactions. Typically sequence driven interactions occur on the Watson-Crick face or Hoogsteen face of the nucleotide. The hybridization of two nucleic acids is affected by a number of conditions and parameters known to those of skill in the art. For example, the salt concentrations, pH, and temperature of the reaction all affect whether two nucleic acid molecules will hybridize.

Parameters for selective hybridization between two nucleic acid molecules are well known to those of skill in the art. For example, in some embodiments selective hybridization conditions can be defined as stringent hybridization conditions. For example, stringency of hybridization is controlled by both temperature and salt concentration of either or both of the hybridization and washing steps. For example, the conditions of hybridization to achieve selective hybridization can involve hybridization in high ionic strength solution (6X SSC or 6X SSPE) at a temperature that is about 12-25°C below the T_m (the melting temperature at which half of the molecules dissociate from their hybridization partners) followed by washing at a combination of temperature and salt concentration chosen so that the washing temperature is about 5°C to 20°C below the T_m . The temperature and salt conditions are readily determined empirically in preliminary experiments in which samples of reference DNA immobilized on filters are hybridized to a labeled nucleic acid of interest and then washed under conditions of different stringencies. Hybridization temperatures are typically higher for DNA-RNA and RNA-RNA hybridizations. The conditions can be used as described above to achieve stringency, or as is known in the art. (Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989; Kunkel et al. *Methods Enzymol.* 1987:154:367, 1987 which is herein incorporated by reference for material at least related to hybridization of nucleic acids). A preferable stringent hybridization condition for a DNA:DNA hybridization can be at about 68°C (in aqueous solution) in 6X SSC or 6X SSPE followed by washing at 68°C. Stringency of hybridization and washing, if desired, can be reduced accordingly as the degree of complementarity desired is decreased, and further,

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depending upon the G-C or A-T richness of any area wherein variability is searched for. Likewise, stringency of hybridization and washing, if desired, can be increased accordingly as homology desired is increased, and further, depending upon the G-C or A-T richness of any area wherein high homology is desired, all as known in the art.

5 Another way to define selective hybridization is by looking at the amount (percentage) of one of the nucleic acids bound to the other nucleic acid. For example, in some embodiments selective hybridization conditions would be when at least about, 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the limiting nucleic acid is bound to the non-limiting nucleic acid. Typically, the non-limiting primer is in for example, 10 or 100 or 1000 fold excess.

10 This type of assay can be performed at under conditions where both the limiting and non-limiting primer are for example, 10 fold or 100 fold or 1000 fold below their K_d , or where only one of the nucleic acid molecules is 10 fold or 100 fold or 1000 fold or where one or both nucleic acid molecules are above their K_d .

Another way to define selective hybridization is by looking at the percentage of primer that gets enzymatically manipulated under conditions where hybridization is required to promote the desired enzymatic manipulation. For example, in some embodiments selective hybridization conditions would be when at least

15 about, 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the primer is enzymatically manipulated under conditions which promote the enzymatic manipulation, for example if the enzymatic manipulation is DNA extension, then selective hybridization conditions would be when at least about 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82,

20 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the primer molecules are extended. Preferred conditions also include those indicated by the manufacturer or indicated in the art as being appropriate for the enzyme performing the manipulation.

Just as with homology, it is understood that there are a variety of methods herein disclosed for determining the level of hybridization between two nucleic acid molecules. It is understood that these

25 methods and conditions can provide different percentages of hybridization between two nucleic acid molecules, but unless otherwise indicated meeting the parameters of any of the methods would be sufficient. For example if 80% hybridization was required and as long as hybridization occurs within the required parameters in any one of these methods it is considered disclosed herein.

It is understood that those of skill in the art understand that if a composition or method meets any one

30 of these criteria for determining hybridization either collectively or singly it is a composition or method that is disclosed herein.

c) Nucleic acids

There are a variety of molecules disclosed herein that are nucleic acid based, including for example the nucleic acids that encode, for example the purinergic receptors, as well as various functional nucleic acids.

35 The disclosed nucleic acids are made up of, for example, nucleotides, nucleotide analogs, or nucleotide substitutes. Non-limiting examples of these and other molecules are discussed herein. It is understood that for example, when a vector is expressed in a cell that the expressed mRNA will typically be made up of A, C, G,

and U. Likewise, it is understood that if, for example, an antisense molecule is introduced into a cell or cell environment through for example exogenous delivery, it is advantageous that the antisense molecule be made up of nucleotide analogs that reduce the degradation of the antisense molecule in the cellular environment.

(1) Nucleotides and related molecules

5 A nucleotide is a molecule that contains a base moiety, a sugar moiety and a phosphate moiety. Nucleotides can be linked together through their phosphate moieties and sugar moieties creating an internucleoside linkage. The base moiety of a nucleotide can be adenin-9-yl (A), cytosin-1-yl (C), guanin-9-yl (G), uracil-1-yl (U), and thymine-1-yl (T). The sugar moiety of a nucleotide is a ribose or a deoxyribose. The phosphate moiety of a nucleotide is pentavalent phosphate. A non-limiting example of a nucleotide would be
10 3'-AMP (3'-adenosine monophosphate) or 5'-GMP (5'-guanosine monophosphate).

A nucleotide analog is a nucleotide, which contains some type of modification to the base, sugar, or phosphate moieties. Modifications to nucleotides are well known in the art and would include for example, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, and 2-aminoadenine as well as modifications at the sugar or phosphate moieties.

15 Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

20 It is also possible to link other types of molecules (conjugates) to nucleotides or nucleotide analogs to enhance for example, cellular uptake. Conjugates can be chemically linked to the nucleotide or nucleotide analogs. Such conjugates include but are not limited to lipid moieties such as a cholesterol moiety. (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989,86, 6553-6556),

A Watson-Crick interaction is at least one interaction with the Watson-Crick face of a nucleotide,
25 nucleotide analog, or nucleotide substitute. The Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute includes the C2, N1, and C6 positions of a purine based nucleotide, nucleotide analog, or nucleotide substitute and the C2, N3, C4 positions of a pyrimidine based nucleotide, nucleotide analog, or nucleotide substitute.

A Hoogsteen interaction is the interaction that takes place on the Hoogsteen face of a nucleotide or
30 nucleotide analog, which is exposed in the major groove of duplex DNA. The Hoogsteen face includes the N7 position and reactive groups (NH₂ or O) at the C6 position of purine nucleotides.

(2) Nucleotide analogs and related molecules

A nucleotide analog is a nucleotide, which contains some type of modification to the base, sugar, or phosphate moieties. Modifications to the base moiety would include natural and synthetic modifications of A,
35 C, G, and T/U as well as different purine or pyrimidine bases, such as uracil-5-yl (.psi.), hypoxanthin-9-yl (I), and 2-aminoadenin-9-yl. A modified base includes but is not limited to 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of

adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Additional base modifications can be found for example in U.S. Pat. No. 3,687,808, Englisch et al., *Angewandte Chemie, International Edition*, 1991, 30, 613, and Sanghvi, Y. S., Chapter 15, *Antisense Research and Applications*, pages 289-302, Crooke, S. T. and Lebleu, B. ed., CRC Press, 1993. Certain nucleotide analogs, such as 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine can increase the stability of duplex formation. Often time base modifications can be combined with for example a sugar modification, such as 2'-O-methoxyethyl, to achieve unique properties such as increased duplex stability. There are numerous United States patents such as 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121; 5,596,091; 5,614,617; and 5,681,941, which detail and describe a range of base modifications. Each of these patents is herein incorporated by reference.

Nucleotide analogs can also include modifications of the sugar moiety. Modifications to the sugar moiety would include natural modifications of the ribose and deoxy ribose as well as synthetic modifications. Sugar modifications include but are not limited to the following modifications at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C₁ to C₁₀, alkyl or C₂ to C₁₀ alkenyl and alkynyl. 2' sugar modifications also include but are not limited to -O[(CH₂)_nO]_mCH₃, -O(CH₂)_nOCH₃, -O(CH₂)_nNH₂, -O(CH₂)_nCH₃, -O(CH₂)_n-ONH₂, and -O(CH₂)_nON[(CH₂)_nCH₃]₂, where n and m are from 1 to about 10.

Other modifications at the 2' position include but are not limited to: C₁ to C₁₀ lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. Similar modifications can also be made at other positions on the sugar, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Modified sugars would also include those that contain modifications at the bridging ring oxygen, such as CH₂ and S. Nucleotide sugar analogs can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. There are numerous United States patents that teach the preparation of such modified sugar structures such as 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, each of which is herein incorporated by reference in its entirety.

Nucleotide analogs can also be modified at the phosphate moiety. Modified phosphate moieties include but are not limited to those that can be modified so that the linkage between two nucleotides contains a phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl and other alkyl phosphonates including 3'-alkylene phosphonate and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates. It is understood that these phosphate or modified phosphate linkage between two nucleotides can be through a 3'-5' linkage or a 2'-5' linkage, and the linkage can contain inverted polarity such as 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included. Numerous United States patents teach how to make and use nucleotides containing modified phosphates and include but are not limited to, 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference.

It is understood that nucleotide analogs need only contain a single modification, but can also contain multiple modifications within one of the moieties or between different moieties.

Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

Nucleotide substitutes are nucleotides or nucleotide analogs that have had the phosphate moiety and/or sugar moieties replaced. Nucleotide substitutes do not contain a standard phosphorus atom. Substitutes for the phosphate can be for example, short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones, formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts. Numerous United States patents disclose how to make and use these types of phosphate replacements and include but are not limited to 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

It is also understood in a nucleotide substitute that both the sugar and the phosphate moieties of the nucleotide can be replaced, by for example an amide type linkage (aminoethylglycine) (PNA). United States

patents 5,539,082; 5,714,331; and 5,719,262 teach how to make and use PNA molecules, each of which is herein incorporated by reference. (See also Nielsen et al., *Science*, 1991, 254, 1497-1500).

It is also possible to link other types of molecules (conjugates) to nucleotides or nucleotide analogs to enhance for example, cellular uptake. Conjugates can be chemically linked to the nucleotide or nucleotide analogs. Such conjugates include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., *Proc. Natl. Acad. Sci. USA*, 1989, 86, 6553-6556), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, 660, 306-309; Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, 1991, 10, 1111-1118; Kabanov et al., *FEBS Lett.*, 1990, 259, 327-330; Svinarchuk et al., *Biochimie*, 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651-3654; Shea et al., *Nucl. Acids Res.*, 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-oxcholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, 277, 923-937. Numerous United States patents teach the preparation of such conjugates and include, but are not limited to U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717; 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241; 5,391,723; 5,416,203; 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, each of which is herein incorporated by reference.

A Watson-Crick interaction is at least one interaction with the Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute. The Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute includes the C2, N1, and C6 positions of a purine based nucleotide, nucleotide analog, or nucleotide substitute and the C2, N3, C4 positions of a pyrimidine based nucleotide, nucleotide analog, or nucleotide substitute.

A Hoogsteen interaction is the interaction that takes place on the Hoogsteen face of a nucleotide or nucleotide analog, which is exposed in the major groove of duplex DNA. The Hoogsteen face includes the N7 position and reactive groups (NH₂ or O) at the C6 position of purine nucleotides.

(3) Sequences

There are a variety of sequences related to the purinergic receptors having the following Genbank Accession Numbers and these sequences and others are herein incorporated by reference in their entireties as well as for individual subsequences contained therein.

There are many sequences of the PX2 receptor, some of which can be found for example herein and others which can be found at Genbank, all of which are herein incorporated by reference. It is understood that the description related to this sequence is applicable to any sequence related to purinergic receptors, for example, unless specifically indicated otherwise. Those of skill in the art understand how to resolve sequence discrepancies and differences and to adjust the compositions and methods relating to a particular sequence to other related sequences. Primers and/or probes can be designed for any of the purinergic receptor sequences given the information disclosed herein and known in the art.

(4) Primers and probes

Disclosed are compositions including primers and probes, which are capable of interacting with the purinergic receptors as disclosed herein. In certain embodiments the primers are used to support DNA amplification reactions. Typically the primers will be capable of being extended in a sequence specific manner. Extension of a primer in a sequence specific manner includes any methods wherein the sequence and/or composition of the nucleic acid molecule to which the primer is hybridized or otherwise associated directs or influences the composition or sequence of the product produced by the extension of the primer. Extension of the primer in a sequence specific manner therefore includes, but is not limited to, PCR, DNA sequencing, DNA extension, DNA polymerization, RNA transcription, or reverse transcription. Techniques and conditions that amplify the primer in a sequence specific manner are preferred. In certain embodiments the primers are used for the DNA amplification reactions, such as PCR or direct sequencing. It is understood that in certain embodiments the primers can also be extended using non-enzymatic techniques, where for example, the nucleotides or oligonucleotides used to extend the primer are modified such that they will chemically react to extend the primer in a sequence specific manner. Typically the disclosed primers hybridize with a purinergic receptor nucleic acid or region of the purinergic receptor nucleic acid or they hybridize with the complement of the purinergic receptor nucleic acid or complement of a region of the purinergic receptor nucleic acid.

d) Delivery of the compositions to cells

(1) Nucleic Acid Delivery

There are a number of compositions and methods which can be used to deliver nucleic acids to cells, either in vitro or in vivo. These methods and compositions can largely be broken down into two classes: viral based delivery systems and non-viral based delivery systems. For example, the nucleic acids can be delivered through a number of direct delivery systems such as, electroporation, lipofection, calcium phosphate precipitation, plasmids, viral vectors, viral nucleic acids, phage nucleic acids, phages, cosmids, or via transfer of genetic material in cells or carriers such as cationic liposomes. Appropriate means for transfection, including viral vectors, chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example, Wolff, J. A., et al., Science, 247, 1465-1468, (1990); and Wolff, J. A. Nature, 352, 815-818, (1991). Such methods are well known in the art and readily adaptable for use with the compositions and methods described herein. In certain cases, the methods will be modified to specifically function with large DNA molecules. Further, these methods can be used to target certain diseases and cell populations by using the targeting characteristics of the carrier.

In the methods described herein, which include the administration and uptake of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), the disclosed nucleic acids can be in the form of naked DNA or RNA, or the nucleic acids can be in a vector for delivering the nucleic acids to the cells, whereby the encoding DNA or DNA or fragment is under the transcriptional regulation of a promoter, as would be well understood by one of ordinary skill in the art as well as enhancers. The vector can be a commercially available preparation, such as an adenovirus vector (Quantum Biotechnologies, Inc. (Laval, Quebec, Canada).

As one example, vector delivery can be via a viral system, such as a retroviral vector system which can package a recombinant retroviral genome (see e.g., Pastan et al., *Proc. Natl. Acad. Sci. U.S.A.* 85:4486, 1988; Miller et al., *Mol. Cell. Biol.* 6:2895, 1986). The recombinant retrovirus can then be used to infect and thereby deliver to the infected cells nucleic acid encoding a broadly neutralizing antibody (or active fragment thereof). The exact method of introducing the altered nucleic acid into mammalian cells is, of course, not limited to the use of retroviral vectors. Other techniques are widely available for this procedure including the use of adenoviral vectors (Mitani et al., *Hum. Gene Ther.* 5:941-948, 1994), adeno-associated viral (AAV) vectors (Goodman et al., *Blood* 84:1492-1500, 1994), lentiviral vectors (Naidini et al., *Science* 272:263-267, 1996), pseudotyped retroviral vectors (Agrawal et al., *Exper. Hematol.* 24:738-747, 1996). Physical transduction techniques can also be used, such as liposome delivery and receptor-mediated and other endocytosis mechanisms (see, for example, Schwartzenberger et al., *Blood* 87:472-478, 1996). The disclosed compositions and methods can be used in conjunction with any of these or other commonly used gene transfer methods.

As one example, if the antibody-encoding nucleic acid or some other nucleic acid encoding a purinergic receptor interactions is delivered to the cells of a subject in an adenovirus vector, the dosage for administration of adenovirus to humans can range from about 10^7 to 10^9 plaque forming units (pfu) per injection but can be as high as 10^{12} pfu per injection (Crystal, *Hum. Gene Ther.* 8:985-1001, 1997; Alvarez and Curiel, *Hum. Gene Ther.* 8:597-613, 1997). A subject can receive a single injection, or, if additional injections are necessary, they can be repeated at six-month intervals (or other appropriate time intervals, as determined by the skilled practitioner) for an indefinite period and/or until the efficacy of the treatment has been established.

Parenteral administration of the nucleic acid or vector, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein. For additional discussion of suitable formulations and various routes of administration of therapeutic compounds, see, e.g., *Remington: The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995.

Nucleic acids that are delivered to cells which are to be integrated into the host cell genome, typically contain integration sequences. These sequences are often viral related sequences, particularly when viral

based systems are used. These viral integration systems can also be incorporated into nucleic acids which are to be delivered using a non-nucleic acid based system of deliver, such as a liposome, so that the nucleic acid contained in the delivery system can be come integrated into the host genome.

Other general techniques for integration into the host genome include, for example, systems designed to promote homologous recombination with the host genome. These systems typically rely on sequence flanking the nucleic acid to be expressed that has enough homology with a target sequence within the host cell genome that recombination between the vector nucleic acid and the target nucleic acid takes place, causing the delivered nucleic acid to be integrated into the host genome. These systems and the methods necessary to promote homologous recombination are known to those of skill in the art.

(2) Non-nucleic acid based systems

The disclosed compositions can be delivered to the target cells in a variety of ways. For example, the compositions can be delivered through electroporation, or through lipofection, or through calcium phosphate precipitation. The delivery mechanism chosen will depend in part on the type of cell targeted and whether the delivery is occurring for example *in vivo* or *in vitro*.

Thus, the compositions can comprise, in addition to the disclosed compositions or vectors for example, lipids such as liposomes, such as cationic liposomes (e.g., DOTMA, DOPE, DC-cholesterol) or anionic liposomes. Liposomes can further comprise proteins to facilitate targeting a particular cell, if desired. Administration of a composition comprising a compound and a cationic liposome can be administered to the blood afferent to a target organ or inhaled into the respiratory tract to target cells of the respiratory tract. Regarding liposomes, see, e.g., Brigham et al. *Am. J. Resp. Cell. Mol. Biol.* 1:95-100 (1989); Felgner et al. *Proc. Natl. Acad. Sci USA* 84:7413-7417 (1987); U.S. Pat. No.4,897,355. Furthermore, the compound can be administered as a component of a microcapsule that can be targeted to specific cell types, such as macrophages, or where the diffusion of the compound or delivery of the compound from the microcapsule is designed for a specific rate or dosage.

In the methods described above which include the administration and uptake of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), delivery of the compositions to cells can be via a variety of mechanisms. As one example, delivery can be via a liposome, using commercially available liposome preparations such as LIPOFECTIN, LIPOFECTAMINE (GIBCO-BRL, Inc., Gaithersburg, MD), SUPERFECT (Qiagen, Inc. Hilden, Germany) and TRANSFECTAM (Promega Biotec, Inc., Madison, WI), as well as other liposomes developed according to procedures standard in the art. In addition, the nucleic acid or vector can be delivered *in vivo* by electroporation, the technology for which is available from Genetronics, Inc. (San Diego, CA) as well as by means of a SONOPORATION machine (ImaRx Pharmaceutical Corp., Tucson, AZ).

The materials can be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These can be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Senter, et al., *Bioconjugate Chem.*, 2:447-451, (1991); Bagshawe, K.D., *Br. J. Cancer*,

60:275-281, (1989); Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie, Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol., 42:2062-2065, (1991)).

These techniques can be used for a variety of other specific cell types. Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of receptor-mediated endocytosis have been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

(3) *In vivo/ex vivo*

As described above, the compositions can be administered in a pharmaceutically acceptable carrier and can be delivered to the subject's cells *in vivo* and/or *ex vivo* by a variety of mechanisms well known in the art (e.g., uptake of naked DNA, liposome fusion, intramuscular injection of DNA via a gene gun, endocytosis and the like).

If *ex vivo* methods are employed, cells or tissues can be removed and maintained outside the body according to standard protocols well known in the art. The compositions can be introduced into the cells via any gene transfer mechanism, such as, for example, calcium phosphate mediated gene delivery, electroporation, microinjection or proteoliposomes. The transduced cells can then be infused (e.g., in a pharmaceutically acceptable carrier) or homotopically transplanted back into the subject per standard methods for the cell or tissue type. Standard methods are known for transplantation or infusion of various cells into a subject.

e) Expression systems

The nucleic acids that are delivered to cells typically contain expression-controlling systems. For example, the inserted genes in viral and retroviral systems usually contain promoters, and/or enhancers to help control the expression of the desired gene product. A promoter is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and can contain upstream elements and response elements.

(1) Viral Promoters and Enhancers

Preferred promoters controlling transcription from vectors in mammalian host cells can be obtained from various sources, for example, the genomes of viruses such as: polyoma, Simian Virus 40 (SV40), adenovirus, retroviruses, hepatitis-B virus and most preferably cytomegalovirus, or from heterologous mammalian promoters, e.g. beta actin promoter. The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment which also contains the SV40 viral origin of replication (Fiers et al., Nature, 273: 113 (1978)). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment (Greenway, P.J. et al., Gene 18: 355-360 (1982)). Of course, promoters from the host cell or related species also are useful herein.

Enhancer generally refers to a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5' (Laimins, L. et al., Proc. Natl. Acad. Sci. 78: 993 (1981)) or 3' (Lusky, M.L., et al., Mol. Cell Bio. 3:1108 (1983)) to the transcription unit. Furthermore, enhancers can be within an intron (Banerji, J.L. et al., Cell 33:729 (1983)) as well as within the coding sequence itself (Osborne, T.F., et al., Mol. Cell Bio. 4:1293 (1984)). They are usually between 10 and 300 bp in length, and they function in cis. Enhancers function to increase transcription from nearby promoters. Enhancers also often contain response elements that mediate the regulation of transcription. Promoters can also contain response elements that mediate the regulation of transcription. Enhancers often determine the regulation of expression of a gene. While many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, -fetoprotein and insulin), typically one will use an enhancer from a eukaryotic cell virus for general expression. Examples are the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

The promoter and/or enhancer can be specifically activated either by light or specific chemical events which trigger their function. Systems can be regulated by reagents such as tetracycline and dexamethasone. There are also ways to enhance viral vector gene expression by exposure to irradiation, such as gamma irradiation, or alkylating chemotherapy drugs.

In certain embodiments the promoter and/or enhancer regions can act as a constitutive promoter and/or enhancer to maximize expression of the region of the transcription unit to be transcribed. In certain constructs the promoter and/or enhancer region be active in all eukaryotic cell types, even if it is only expressed in a particular type of cell at a particular time. A preferred promoter of this type is the CMV promoter (650 bases). Other preferred promoters are SV40 promoters, cytomegalovirus (full length promoter), and retroviral vector LTR.

It has been shown that all specific regulatory elements can be cloned and used to construct expression vectors that are selectively expressed in specific cell types such as melanoma cells. The glial fibrillary acetic protein (GFAP) promoter has been used to selectively express genes in cells of glial origin.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human or nucleated cells) can also contain sequences necessary for the termination of transcription which can affect

mRNA expression. These regions are transcribed as polyadenylated segments in the untranslated portion of the mRNA encoding tissue factor protein. The 3' untranslated regions also include transcription termination sites. It is preferred that the transcription unit also contains a polyadenylation region. One benefit of this region is that it increases the likelihood that the transcribed unit will be processed and transported like mRNA.

5 The identification and use of polyadenylation signals in expression constructs is well established. It is preferred that homologous polyadenylation signals be used in the transgene constructs. In certain transcription units, the polyadenylation region is derived from the SV40 early polyadenylation signal and consists of about 400 bases. It is also preferred that the transcribed units contain other standard sequences alone or in combination with the above sequences improve expression from, or stability of, the construct.

10 (2) Markers

The vectors can include nucleic acid sequence encoding a marker product. This marker product is used to determine if the gene has been delivered to the cell and once delivered is being expressed. Preferred marker genes are the *E. coli* lacZ gene, which encodes β -galactosidase, and green fluorescent protein.

In some embodiments the marker can be a selectable marker. Examples of suitable selectable
15 markers for mammalian cells are dihydrofolate reductase (DHFR), thymidine kinase, neomycin, neomycin analog G418, hygromycin, and puromycin. When such selectable markers are successfully transferred into a mammalian host cell, the transformed mammalian host cell can survive if placed under selective pressure. There are two widely used distinct categories of selective regimes. The first category is based on a cell's metabolism and the use of a mutant cell line which lacks the ability to grow independent of a supplemented
20 media. Two examples are: CHO DHFR- cells and mouse LTK- cells. These cells lack the ability to grow without the addition of such nutrients as thymidine or hypoxanthine. Because these cells lack certain genes necessary for a complete nucleotide synthesis pathway, they cannot survive unless the missing nucleotides are provided in a supplemented media. An alternative to supplementing the media is to introduce an intact DHFR or TK gene into cells lacking the respective genes, thus altering their growth requirements. Individual cells
25 which were not transformed with the DHFR or TK gene will not be capable of survival in non-supplemented media.

The second category is dominant selection which refers to a selection scheme used in any cell type and does not require the use of a mutant cell line. These schemes typically use a drug to arrest growth of a host cell. Those cells which have a novel gene would express a protein conveying drug resistance and would
30 survive the selection. Examples of such dominant selection use the drugs neomycin, (Southern P. and Berg, P., *J. Molec. Appl. Genet.* 1:327 (1982)), mycophenolic acid, (Mulligan, R.C. and Berg, P. *Science* 209:1422 (1980)) or hygromycin, (Sugden, B. et al., *Mol. Cell. Biol.* 5:410-413 (1985)). The three examples employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid) or hygromycin, respectively. Others include the neomycin analog G418
35 and puromycin.

f) Peptides

(1) Protein variants

As discussed herein there are numerous variants of the purinergic receptor proteins and that are known and herein contemplated. In addition, to the known functional purinergic receptor species variants there are derivatives of the purinergic receptor proteins which also function in the disclosed methods and compositions. Protein variants and derivatives are well understood to those of skill in the art and in can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Immunogenic fusion protein derivatives, such as those described in the examples, are made by fusing a polypeptide sufficiently large to confer immunogenicity to the target sequence by cross-linking in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. These variants ordinarily are prepared by site-specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions preferably are made in adjacent pairs, i.e. a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof can be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the following Tables 5 and 6 and are referred to as conservative substitutions.

TABLE 5: Amino Acid Abbreviations

Amino Acid	Abbreviations
Alanine	AlaA
Allosoleucine	Aile
Arginine	ArgR
Asparagines	AsnN
Aspartic acid	AspD
Cysteine	CysC
Glutamic acid	GluE
Glutamine	GlnQ
Glycine	GlyG
Histidine	HisH
Isoleucine	IleI
Leucine	LeuL

Amino Acid	Abbreviations
Lysine	LysK
Phenylalanine	PheF
Proline	ProP
Pyroglutamic acid	Glu
Serine	SerS
Threonine	ThrT
Tyrosine	TyrY
Tryptophan	TrpW
Valine	ValV

Table 6.

Original Residue Exemplary Conservative Substitutions, others are known in the art.

	Ala	ser
	Arg	lys, gln
5	Asn	gln; his
	Asp	glu
	Cys	ser
	Gln	asn, lys
	Glu	asp
10	Gly	ala
	His	asn;gln
	Ile	leu; val
	Leu	ile; val
	Lys	arg; gln;
15	Met	Leu; ile
	Phe	met; leu; tyr
	Ser	thr
	Thr	ser
	Trp	tyr
20	Tyr	trp; phe
	Val	ile; leu

Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those in Table 6, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

For example, the replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are included within the mosaic polypeptides provided herein.

Substitutional or deletional mutagenesis can be employed to insert sites for N-glycosylation (Asn-X-Thr/Ser) or O-glycosylation (Ser or Thr). Deletions of cysteine or other labile residues also can be desirable. Deletions or substitutions of potential proteolysis sites, e.g. Arg, is accomplished for example by deleting one of the basic residues or substituting one by glutamyl or histidyl residues.

5 Certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutamyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and asparyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the o-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, *Proteins: Structure and Molecular Properties*, W. H. Freeman & Co., San Francisco pp 79-86 [1983]), acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl.

15 It is understood that one way to define the variants and derivatives of the disclosed proteins herein is through defining the variants and derivatives in terms of homology/identity to specific known sequences. For example, SEQ ID NO:1 sets forth a particular sequence of a P2X receptor. Specifically disclosed are variants of these and other proteins herein disclosed which have at least, 70% or 75% or 80% or 85% or 90% or 95% homology to the stated sequence. Those of skill in the art readily understand how to determine the homology of two proteins. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

20 Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48: 443 (1970), by the search for similarity method of Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

25 The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989 which are herein incorporated by reference for at least material related to nucleic acid alignment.

30 It is understood that the description of conservative mutations and homology can be combined together in any combination, such as embodiments that have at least 70% homology to a particular sequence wherein the variants are conservative mutations.

35 As this specification discusses various proteins and protein sequences it is understood that the nucleic acids that can encode those protein sequences are also disclosed. This would include all degenerate sequences related to a specific protein sequence, i.e. all nucleic acids having a sequence that encodes one particular protein sequence as well as all nucleic acids, including degenerate nucleic acids, encoding the disclosed

variants and derivatives of the protein sequences. Thus, while each particular nucleic acid sequence cannot be written out herein, it is understood that each and every sequence is in fact disclosed and described herein through the disclosed protein sequence. It is also understood that while no amino acid sequence indicates what particular DNA sequence encodes that protein within an organism, where particular variants of a disclosed protein are disclosed herein, the known nucleic acid sequence that encodes that protein in the particular organism from which that protein arises is also known and herein disclosed and described.

g) **Pharmaceutical carriers/Delivery of pharmaceutical products**

As described herein, the compositions, such as the ATP and ATP analogs, can also be administered *in vivo* in a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material can be administered to a subject, along with the nucleic acid or vector, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art.

The compositions can be administered orally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally, topically or the like, including topical intranasal administration or administration by inhalant. As used herein, "topical intranasal administration" means delivery of the compositions into the nose and nasal passages through one or both of the nares and can comprise delivery by a spraying mechanism or droplet mechanism, or through aerosolization of the composition. Administration of the compositions by inhalant can be through the nose or mouth via delivery by a spraying or droplet mechanism. Delivery can also be directly to any area of the respiratory system (e.g., lungs) via intubation. The exact amount of the compositions required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the allergic disorder being treated, the particular nucleic acid or vector used, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every composition. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

Parenteral administration of the composition, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein.

The materials can be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These can be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Senter, et al., Bioconjugate Chem., 2:447-451, (1991); Bagshawe, K.D., Br. J. Cancer, 60:275-281, (1989); Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie,

Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol, 42:2062-2065, (1991)).

Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The

5 following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell

10 surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of

15 receptor-mediated endocytosis have been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

(1) Pharmaceutically Acceptable Carriers

Suitable carriers and their formulations are described in *Remington: The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995. Typically, an

20 appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carrier include, but are not limited to, saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the

25 form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers can be more preferable depending upon, for instance, the route of administration and concentration of composition being administered.

Pharmaceutical carriers are known to those skilled in the art. These most typically would be standard carriers for administration of drugs to humans, including solutions such as sterile water, saline, and buffered

30 solutions at physiological pH. The compositions can be administered intramuscularly or subcutaneously. Other compounds will be administered according to standard procedures used by those skilled in the art.

Pharmaceutical compositions can include carriers, thickeners, diluents, buffers, preservatives, surface-active agents and the like in addition to the molecule of choice. Pharmaceutical compositions can also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like.

35 The pharmaceutical composition can be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration can be topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous

drip, subcutaneous, intraperitoneal or intramuscular injection. The disclosed antibodies can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, or transdermally.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives can also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

Formulations for topical administration can include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like can be necessary or desirable.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders can be desirable.

Some of the compositions can potentially be administered as a pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

(2) Therapeutic Uses

Effective dosages and schedules for administering the compositions can be determined empirically, and making such determinations is within the skill in the art. The dosage ranges for the administration of the compositions are those large enough to produce the desired effect in which the symptoms of the disorder are affected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient, route of administration, or whether other drugs are included in the regimen, and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any counterindications. Dosage can vary, and can be administered in one or more dose administrations hourly or daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. For example, guidance in selecting appropriate doses for antibodies can be found in the literature on therapeutic uses of antibodies, e.g., Handbook of Monoclonal Antibodies, Ferrone et al., eds., Noyes Publications, Park Ridge, N.J., (1985) ch. 22 and pp. 303-357; Smith et al., Antibodies in Human Diagnosis and Therapy, Haber et al., eds., Raven Press, New York (1977) pp. 365-389. A typical

daily dosage of the ATP or ATP analogs used alone might range from about 1 $\mu\text{g/kg}$ to up to 100 mg/kg of body weight or more per day, depending on the factors mentioned above. For example, based on the similarities of EC_{50} concentrations for P2 receptors across many species an effective dose to modulate smell in a human would be similar to our mouse model, i.e., 10-200 μM .

5 Following administration of a disclosed composition, such as an ATP analog, for the modulation of smell, the efficacy of the therapeutic composition can be assessed in various ways well known to the skilled practitioner. For instance, one of ordinary skill in the art will understand that the compositions disclosed herein are efficacious in modulating, such as enhancing or reducing the sensation of smell in a subject, by observing that the composition reduces or enhances the sensation of smell to a particular or general odor
10 stimulant. Smell sensation can be measured by methods that are known in the art, for example, and in vitro methods using an ORN calcium imaging assay as discussed herein, can also be used.

 The compositions that modulate smell disclosed herein can be administered prophylactically to patients or subjects who are at risk for being exposed to severe or damaging odor stimulation or who have a desire to have either an increased or decreased sensitivity to an odor. For example, elderly people can increase
15 sensitivity to odor to compensate for loss during aging. Conversely, those on chemotherapy drugs may need decreased odor sensitivity to reduce nausea.

 Other molecules that modulate odor sensitivity, but do not have a particular pharmaceutical function can be used for tracking changes within ORNs.

h) Computer readable mediums

20 It is understood that the disclosed nucleic acids and proteins and compositions can be represented as a sequence consisting of the nucleotides of amino acids. There are a variety of ways to display these sequences, for example the nucleotide guanosine can be represented by G or g. Likewise the amino acid valine can be represented by Val or V. Those of skill in the art understand how to display and express any nucleic acid or protein sequence in any of the variety of ways that exist, each of which is considered herein disclosed.
25 Specifically contemplated herein is the display of these sequences and ATP or ATP analogs on computer readable mediums, such as, commercially available floppy disks, tapes, chips, hard drives, compact disks, and videodisks, or other computer readable mediums. Also disclosed are the binary code representations of the disclosed sequences and ATP or ATP analogs. Those of skill in the art understand what computer readable mediums. Thus, computer readable mediums on which the nucleic acids or protein sequences are recorded,
30 stored, or saved

i) Chips and micro arrays

 Disclosed are chips where at least one address is the sequences or part of the sequences set forth in any of the nucleic acid sequences disclosed herein. Also disclosed are chips where at least one address is the sequences or portion of sequences set forth in any of the peptide sequences disclosed herein. Disclosed are
35 chips where at least one address is the composition, such as an ATP analog, disclosed herein.

 Also disclosed are chips where at least one address is a variant of the sequences or part of the sequences set forth in any of the nucleic acid sequences disclosed herein. Also disclosed are chips where at

least one address is a variant of the sequences or portion of sequences set forth in any of the peptide sequences disclosed herein.

j) Kits

Disclosed herein are kits that are drawn to reagents that can be used in practicing the methods disclosed herein. The kits can include any reagent or combination of reagent discussed herein or that would be understood to be required or beneficial in the practice of the disclosed methods. For example, the kits could include an ATP analog in a formulation for delivery to an ORN. For example, disclosed is a kit for modulating odor sensitivity comprising ATP in a formulation for delivery to an ORN.

D. Methods of making the compositions

The compositions disclosed herein and the compositions necessary to perform the disclosed methods can be made using any method known to those of skill in the art for that particular reagent or compound unless otherwise specifically noted.

1. ATP analog generation

The disclosed ATP analogs can be made using a variety of synthetic procedures. Often the analogs can be purchased. For example, the following analogs can be purchased from Sigma Inc., 2-(Methylthio)adenosine 5'-triphosphate, 2-Chloroadenosine 5' triphosphate, 2',5'-Dideoxyadenosine 3'-triphosphate, 2',3'-O-(4-Benzoylbenzoyl)adenosine 5'-triphosphate, 2'-Monophosphoadenosine 5'-diphosphoribose, ATP-Ribose, 8-Bromoadenosine 5'-triphosphate, Adenosine 5'-triphosphate P3-[1-(2-nitrophenyl)ethyl ester] (Caged ATP/NPE caged ATP), Adenosine 5'-triphosphate, Adenosine 5'-(β , γ -imido)triphosphate, Adenosine 5'-[γ -thio]triphosphate, Adenosine 5'-triphosphate g-(1-[2-nitrophenyl]ethyl ester) (Caged ATP), 125229-58-5 minimum 95%, and 2',3'-O-(2,4,6-Trinitrophenyl) adenosine 5'-triphosphate. It is understood that these compositions and others come as salts, such as lithium, or sodium salts, as well as, for example, triethylammonium salts, and that they can also be formulated in appropriate pharmaceutical salts.

2. Nucleic acid synthesis

For example, the nucleic acids, such as, the oligonucleotides to be used as primers can be made using standard chemical synthesis methods or can be produced using enzymatic methods or any other known method. Such methods can range from standard enzymatic digestion followed by nucleotide fragment isolation (see for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd Edition (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989) Chapters 5, 6) to purely synthetic methods, for example, by the cyanoethyl phosphoramidite method using a Milligen or Beckman System 1Plus DNA synthesizer (for example, Model 8700 automated synthesizer of Milligen-Bioscience, Burlington, MA or ABI Model 380B). Synthetic methods useful for making oligonucleotides are also described by Ikuta *et al.*, *Ann. Rev. Biochem.* 53:323-356 (1984), (phosphotriester and phosphite-triester methods), and Narang *et al.*, *Methods Enzymol.*, 65:610-620 (1980), (phosphotriester method). Protein nucleic acid molecules can be made using known methods such as those described by Nielsen *et al.*, *Bioconjug. Chem.* 5:3-7 (1994).

E. Methods of using the compositions

1. Methods of using the compositions as research tools

The disclosed compositions can be used in a variety of ways as research tools. For example, the disclosed compositions, such as ATP and analogs, can be used to study the signal transduction pathways related to olfactory signaling.

The disclosed compositions can be used as discussed herein as either reagents in micro arrays or as reagents to probe or analyze existing microarrays. The compositions can also be used in any known method of screening assays, related to chip/micro arrays. The compositions can also be used in any known way of using the computer readable embodiments of the disclosed compositions, for example, to study relatedness or to perform molecular modeling analysis related to the disclosed compositions.

a) Screening assays

Provided are methods of screening for an agonist or an antagonist of purinergic receptor of the olfactory system, comprising contacting a purinergic receptor with a test compound; detecting intracellular calcium levels; and screening for a change in calcium levels as compared to a control level, a change indicating the compound is an agonist or an antagonist of the olfactory system.

Screening optionally takes place in multi-well plates. Multi-well plates are standard in the art and come in a variety of sizes and shapes. For example, the multi-well plate can be 24, 48, or 96 well plates. Such screening assays can be automated or further modified for high throughput analysis. For high throughput screening, each well can include numerous test components. If a positive reaction is detected in a well, the screening is repeated with one of the test compounds contained in a single well.

An "elevation in calcium" is defined as an increase in calcium levels greater than 1 nM above basal levels. The change in calcium levels can be between 5 nM and 10 nM, for example. The elevation in calcium can also be greater than 100 nM above basal levels. A "transient reduction in calcium" is defined as decrease in calcium levels greater than 1 nM below basal levels. The reduction in calcium can also be greater than 100 nM below basal levels.

The time defined as "transient" means not permanent. Thus, transient can be less than 10 seconds, less than 30 seconds, less than 1 minute, less than 5 minutes, less than 10 minutes, or less than 20 minutes, for example.

The term "sustained" means that the effect continues for a period of time. For example, sustained can be greater than 1 minute, greater than 5 minutes, greater than 10 minutes, greater than an hour, greater than 24 hours, or greater than 1 year.

Also disclosed is a method of screening for an agonist or an antagonist of a purinergic receptor of the olfactory system, comprising contacting a first purinergic receptor expressing cell with a set of test compounds; detecting calcium levels in the first purinergic receptor cell; and selecting each compound in the set that contacted the first purinergic receptor cell, wherein the first purinergic receptor cell showed a transient change in calcium as compared to a control level, indicating the compound is an agonist or an antagonist of a purinergic receptor of the olfactory system. The method can further comprise the steps of contacting a second

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purinergic receptor cell with one test compound selected above, and detecting calcium levels in the second purinergic receptor cell, wherein a transient change in calcium as compared to a control level indicates the compound is an agonist or an antagonist of a purinergic receptor of the olfactory system.

Also disclosed is a method of screening for an agonist or an antagonist of a purinergic receptor of the olfactory system, comprising contacting a test compound with a cell that expresses a heterologous nucleic acid that encodes a purinergic receptor; and detecting calcium levels in the cell; a transient change in calcium as compared to a control level, indicating an agonist or an antagonist of a purinergic receptor of the olfactory system.

Also contemplated are agents identified by the screening methods described herein, as well as methods of making those agents. An example of a method of making an agent includes identifying the agent using the methods provided herein, and manufacturing the agent or manufacturing the agent in a pharmaceutically acceptable carrier.

Preferably, the cell is a cell that lacks the receptor prior to introduction of the heterologous nucleic acid. The cell can be transiently transfected with the heterologous nucleic acid or a stable cell line containing the expressed receptor can be made using standard techniques in the art. By "heterologous nucleic acid" is meant that any heterologous or exogenous nucleic acid can be inserted into a vector for transfer into a cell, tissue or organism. The nucleic acid can encode a polypeptide or protein or an antisense RNA, for example. The nucleic acid can be functionally linked to a promoter. By "functionally linked" is meant such that the promoter can promote expression of the heterologous nucleic acid, as is known in the art, such as appropriate orientation of the promoter relative to the heterologous nucleic acid. Furthermore, the heterologous nucleic acid preferably has all appropriate sequences for expression of the nucleic acid, as known in the art, to functionally encode, i.e., allow the nucleic acid to be expressed. The nucleic acid can include, for example, expression control sequences, such as an enhancer, and necessary information processing sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences.

Calcium levels and changes in calcium levels can be detected using a calcium indicator such as the cell-permeable methyl ester form of Fura-2, which is Fura-2/AM. In another example, a fluorescence plate reader is used that detects a single wavelength, such as Ca^{2+} indicator dyes Fluo 3, Fluo 4 AM, Quin 2, Indo-1 and Indo-4.

Optionally, the compound being screened can augment the effects of other compounds such as ATP, for example. In this case, the compound being screened can be tested in the presence of another compound that stimulates the purinergic receptor. For example, the purinergic receptor expressing cell can be in a solution containing an effective amount of ATP. An "effective amount of ATP" is defined as about 300 nM to about 1 mM of ATP.

F. Examples

Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

1. Example 1 ATP differentially modulates odor responsiveness through purinergic receptor activation: activation of purinergic receptor subtypes differentially modulates odor sensitivity

a) Results

(1) Localization of Purinergic Receptors in the Peripheral Olfactory System

Using RT-PCR and immunohistochemical methods ionotropic P2X₂ and G-protein-coupled P2Y₂ receptor expression were found in both olfactory epithelium and olfactory bulb. RT-PCR analysis revealed mRNA expression for the P2Y₂ receptor and two isoforms of the P2X₂ receptor; P2X₂₋₁ (Brake, A. J., et al., Nature 371, 519-523 (1994)) and P2X₂₋₂ (Brandle et al., 1997) (Fig. 1A). To identify the cell type and subcellular distribution of purinergic receptors in olfactory epithelium and olfactory bulb, antibodies against P2X₁, P2X₂, P2X₄ and P2Y₂ receptors, and olfactory marker protein (OMP), which is found in mature olfactory receptor neurons (ORNs) were used. OMP-positive ORNs showed punctate immunoreactivity (IR) to P2X₁ and P2X₄ antibodies on cell somas and axons (Fig. 1B, C) and P2Y₂-IR on the dendrites, somas and axons (Fig. 1D). Both P2X- and P2Y-IR was absent from dendritic knobs and cilia of ORNs. OMP-negative ORNs and basal cells (olfactory stem cells) showed P2X- and P2Y-IR. Sustentacular cells and Bowman's glands showed only P2Y₂-IR (Fig 1D). In the olfactory bulb, there was P2X₁, P2X₂, P2X₄ and P2Y₂ receptor IR in the olfactory nerve layer, the glomerular layer and the mitral cell layer. There was no P2X₂-IR in the olfactory neuroepithelium; however, there was punctate P2X₂-IR on blood vessels just below the basal cells. Thus, the underlying blood vessels are the likely source of P2X₂ mRNA identified by the RT-PCR studies of the olfactory epithelium. Preabsorption of the primary antibody with peptide antigen (Fig. 1E) or omission of the primary antibody blocked the purinergic receptor staining. Identification of regionally localized purinergic receptors in mammalian olfactory epithelium is consistent with extracellular ATP playing multiple roles in the peripheral olfactory system.

(2) Purinergic Receptors are Functional In Cultured Olfactory Receptor Neurons

The physiological activation of purinergic receptors in cultured mouse ORNs (Vargas and Lucero, 1999a) were examined using both electrophysiology (Danaceau and Lucero, 1998) (Fig. 2A), and calcium imaging (Fig. 2B-E). ATP (10 μM) evoked inward currents in 39% (27/69 ORNs) of the perforated-patched mouse ORNs during brief (1-10 s) applications (Fig. 2A). Some cells exhibited a distinct, long latent period consistent with slowly activating G-protein coupled P2Y receptors (cell 1, Fig. 2A inset) (13/27 cells; latency = 1140 ± 236 ms; I_{max} = -29 ± 8 pA). Rapid activation of inward current that closely followed the ATP stimulus profile with little or no desensitization was also observed, indicating involvement of non-desensitizing ionotropic P2X receptors [P2X₄ and/or P2X₇] (Ralevic and Burnstock, 1998) (Fig. 2A, cell 2)

(14/27 cells; latency = 81 ± 15 ms, $I_{\max} = -235 \pm 74$ pA). These electrophysiological results support the immunohistochemical evidence of expression of both purinergic receptor subtypes in ORNs.

Extracellularly applied ATP evoked a rapid transient increase in $[Ca^{2+}]_i$ (Fig. 2B, C; 76/84 ORNs). On average, ATP (10 μ M) induced a $151 \pm 12\%$ Δ fluorescence (F)/F increase in $[Ca^{2+}]_i$ (n = 76; range 13 - 398%). Averaged dose-response relations for ATP-induced $[Ca^{2+}]_i$ increases in cultured mouse ORNs gave half-maximally effective concentrations (EC_{50}) of 1.6 μ M (n = 58), comparable to previous reports for brain P2X receptors (Fig. 2D) (North and Barnard, 1997; North and Surprenant, 2000; Ralevic and Burnstock, 1998). In the absence of external Ca^{2+} , the ATP-induced rise in $[Ca^{2+}]_i$ was $31 \pm 11\%$ larger than calcium transients in the presence of Ca^{2+} , although in a few ORNs (3/19), the ATP-evoked increases were smaller, but never absent, in Ca^{2+} -free solution (Fig. 2E). The increase in fluorescence intensity in the absence of Ca^{2+} indicates that (1) part of the signal results from release from intracellular Ca^{2+} stores, implicating P2Y receptor activation, and (2) Ca^{2+} can reduce the concentration of free ATP or modulate the purinergic receptor (Honore et al., 1989; North and Surprenant, 2000). Thus, electrophysiology and Ca^{2+} imaging show that purinergic receptors are functional in primary cultures of mouse ORNs.

15 (3) Confocal Imaging of Olfactory Epithelium Slices

To study the effects of ATP on odor responses, acutely prepared slices of mouse olfactory epithelium were used. Confocal imaging of fluo-4 AM-loaded olfactory epithelium slices allows simultaneous recording from identified structures within the olfactory epithelium, i.e., both ORNs and sustentacular cells. Reproducible odor-evoked calcium transients were obtained when imaging ORNs >100 μ m below the surface of the slice (Fig. 3A1-A5). Odor-evoked calcium transients rapidly activated and returned to basal levels within 125.7 ± 11.1 s (Fig. 3A, n = 11 ORNs). Superfusion of ATP (10 μ M) onto this slice evoked calcium transients from all 11 ORNs previously identified by their response to odors, although the responses to ATP (Fig. B1-B4) are less obvious than the odor responses (Fig. 3B2). The difference in robustness can be due to poor access to the ATP stimulus: odorant receptors are on the cilia of the ORNs, which extend beyond the outer edge of the olfactory epithelium, whereas purinergic receptors are located deeper within the olfactory epithelium. ATP also evoked calcium transients from sustentacular cells, identified by their location, morphology, and lack of response to odor (Fig. 3B3). The latency of activation for the ATP-evoked calcium transient was shorter in the ORN (Fig. 3B2, solid up arrowhead) than in sustentacular cells (Fig. 3B3, open down arrowhead). Collectively, this was consistent with ORNs expressing the faster P2X receptors, and the sustentacular cells expressing the slower G-protein coupled P2Y receptors.

To further test whether functional purinergic receptor subtypes are differentially expressed in olfactory epithelium cell types, purinergic receptor agonists were used. As there are no completely specific purinergic receptor agonists (Ralevic and Burnstock, 1998), the selectivity was determined as discussed herein. The P2X 'selective' agonist $\beta\gamma$ -methylene ATP was superfused onto the slice (Fig. 3C). Only the ORNs, and not the sustentacular cells, responded to $\beta\gamma$ -methylene ATP with an increase in $[Ca^{2+}]_i$ (Fig. 3C5). The P2Y 'selective' agonist UTP evoked calcium transients in both ORNs and sustentacular cells (Fig. 3D). However, compared to the non-selective agonist ATP, the peak amplitudes were smaller in the ORNs and the latency of activation in the ORNs was longer and equivalent to the latency of activation in the sustentacular cells

(compare Fig. 3D2,5 to B2,5). These data indicated that the ORN expressed P2X and, to a lesser extent, P2Y receptors, and that the sustentacular cells expressed only P2Y receptors. It further confirms the immunohistochemical and electrophysiological evidence for differential expression of purinergic receptors in mammalian olfactory epithelium.

5 A variety of non-selective purinergic receptor agonists (ATP, ATP γ S, AMP), P2Y-'selective' agonists (UTP, ADP, MeS-ADP), P2X-'selective' agonists (β -methylene ATP) (Fig. 4A, B), and an adenosine receptor 'selective' agonist (adenosine) were superfused onto olfactory epithelial slices and the change in [Ca²⁺]_i was measured. Adenosine- or AMP-evoked calcium transients were never observed. ORNs responded with approximately equal frequency to P2Y and P2X receptor agonists whereas sustentacular cells responded
10 primarily to P2Y receptor agonists. The general non-specific purinergic receptor antagonists Suramin and pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) were used to further confirm that purinergic-evoked calcium transients were mediated via purinergic receptors. In ORNs previously shown to respond to both ATP and UTP, Suramin (100 μ M) reversibly blocked both ATP- and UTP-evoked calcium transients by 88 \pm 2 and 72 \pm 6% (n = 9). PPADS (25 μ M) also reversibly blocked the ATP- and UTP-evoked calcium
15 transients by 87 \pm 5 and 92 \pm 3% (n = 5). Purinergic receptor antagonists also reversibly blocked purinergic nucleotide-evoked calcium transients in sustentacular cells. Suramin blocked ATP- and UTP-evoked Ca²⁺ transients by 90 \pm 1 and 89 \pm 1% (n = 37) and PPADS blocked the transients by 82 \pm 2 and 76 \pm 2%, respectively (n = 30). Collectively, the data show that the ATP-evoked calcium transients were mediated by P2X and P2Y receptors.

20 (4) ATP Modulates Odor Responses

Calcium is an important intracellular messenger during odor transduction affecting signal amplification (Lowe and Gold, 1993) and adaptation (Zufall et al., 1991). The data indicate that purinergic nucleotides evoke robust increases in intracellular calcium. Odors were sequentially superfused, ATP and the combination of odors and ATP onto olfactory epithelium slice preparation. It was found that ATP could (1)
25 have no effect, (2) cause suppression (Fig. 5A), where the calcium transient evoked by co-application is less than the sum of the calcium transients evoked by the individual components, or (3) cause enhancement (Fig. 5B), in which the calcium transient due to co-application is larger than the sum of the individual components. The increases in Ca²⁺ elicited from co-application of ATP and odor from two cells (Fig. 5A, B) have been expressed as a proportion of the sum of the individual responses (Fig. 5C). Of the cells that responded to
30 odor, 62% exhibited a significant decrease in the summed [Ca²⁺]_i increase (mean suppression = 57 \pm 5%; paired t-test, p = 0.01, n = 26). The observed decrease due to co-application was not the result of run-down because post-co-application responses both to ATP and to odors were \pm 10% of pre-co-application (Fig. 5A). Of the odor-responsive ORNs, only two exhibited a >20% increase in evoked [Ca²⁺]_i increase (mean enhancement = 157 \pm 34%), indicating a combined effect. Thus, ATP significantly reduced odor-induced
35 calcium responses in the majority of ORNs.

(5) Activation Of Purinergic Receptor Subtypes Modulates Odor Sensitivity

The observation of both suppressive and combined responses indicates that ATP can modulate odor responses via activation of different purinergic receptor subtypes. This hypothesis was tested by sequentially
5 superfusing odors, various selective purinergic receptor agonists and the combination of odors and purinergic receptor agonists onto the olfactory epithelium slice preparation. We found that co-application of the P2X agonist $\beta\gamma$ -methylene ATP (10 μ M) and odor (1) enhanced the calcium transient amplitude by $168 \pm 44\%$ (2/16 cells; Figures 6A-B), (2) had no effect on amplitude ($15 \pm 1\%$; 2/16 cells; data not shown), or (3) suppressed the amplitude of Ca^{2+} transient by $42 \pm 5\%$ (12/16 cells; Figures 6C-D). Overall, there is a
10 statistically significant $25 \pm 11\%$ reduction in the average amplitude of the Ca^{2+} transient by co-application of odors and $\beta\gamma$ -methylene ATP (16/16 cells; $p < 0.04$, paired Student's t-test), even when including the 2 cells that did not show a significant change and the 2 cells that had an enhanced response. In contrast, co-application of the P2Y agonist ADP β S (10 μ M) and odor suppressed the Ca^{2+} transient amplitude by $41 \pm 4\%$ (15/15 cells; $p < 0.001$, paired Student's t-test; Fig 6E-F). Thus, the P2Y agonist ADP β S reduced the odor
15 responsiveness of ORNs in all cells tested. In contrast, $\beta\gamma$ -methylene ATP, like ATP, enhanced the odor responsiveness due to the activation of P2X receptors in a few cells. However, in the majority of ORNs, the P2X specific agonist significantly reduced odor-induced Ca^{2+} transients.

Disclosed herein, purinergic receptor subtypes are differentially expressed in ORNs and sustentacular cells, and ORNs express multiple purinergic receptor subtypes. In other cell types, expression of more than
20 one type of purinergic receptor allows for regulation of multiple effectors and modification of agonist-evoked responses, and provides a mechanism for rapid and local fine tuning at the cellular level (Ralevic and Burnstock, 1998). Disclosed immunohistochemical studies showed a notable absence of purinergic receptors in the dendritic knobs and cilia, the site of odor transduction, whereas both P2X and P2Y receptors are located on cell somas and other regions. This indicates that purinergic receptor activation is unlikely to affect initial
25 odor-induced receptor potentials, but can shape the final integrated output of the cell. Extracellular purine nucleotides have been reported to exert multiple trophic actions in the central nervous system (Neary et al., 1996). Because the olfactory neuroepithelium is constantly exposed to airborne pollutants and microbes, it continuously regenerates; different populations of neurons are in various stages of development, including birth, maturation, and programmed cell death or apoptosis (Graziadei and Monti-Graziadei, 1978). Thus, ATP
30 released by acutely injured cells could act as an early signal of cell and tissue damage, and, due to the mitogenic and growth-promoting effects of purinergic receptor activation, stimulate regeneration. Growth promotion can be mediated by P2Y receptors, which, like other growth factor receptors, induce a cascade of intracellular events that trigger cell proliferation (Neary et al., 1996).

A longstanding dogma, that odor sensitivity is not modulated at the level of the olfactory epithelium,
35 is based on anatomical studies showing absence of efferent synapses on ORNs (Getchell, 1986; Graziadei, 1971). A recent study showing release of ATP in the olfactory epithelium following noxious stimuli (Kilgour et al., 2000) provides a physiological source for a neuromodulatory substance that does not require efferent innervation.

b) Experimental Procedures**(1) RT-PCR**

Total RNA was isolated from rat olfactory epithelium using Trizol (GIBCO BRL). Polyadenylated mRNA was selected using a cellulose oligo(dT) matrix (QuickPrep® Micro mRNA purification kit, Amersham Pharmacia Biotech). First-strand cDNA was prepared from 40 ng mRNA using SuperScript™ II RNase H- RT
5 according to GIBCO BRL procedures. A control reaction omitting the reverse transcriptase was included to confirm absence of genomic contamination. First strand cDNA was amplified using Platinum®Taq DNA polymerase (Gibco BRL). Primers for detection of P2X₂ transcripts were 756-775 sense and 1537-1558 antisense oligonucleotides (accession #U14414)(Brake et al., 1994), primers for P2Y₂ transcripts were 1288-
10 1307 sense and 1931-1950 antisense oligonucleotides (accession #U09402), primers for β -actin transcripts (Lopez-Candales et al., 1995) were 1038-1067 sense and 1875-1905 antisense oligonucleotides and primers for neuron specific enolase were 348-368 sense and 1101-1123 antisense oligonucleotides (accession #M11931). All the primer pairs (100 μ M) were used with a 30-cycle profile performed as follows: 94°C denaturation (2 min), 96°C denaturation (45 s), 60°C annealing (1 min) and 72°C extension (1.5 min). PCR
15 products were separated and visualized using ethidium bromide-stained agarose gels (1%). A semi-nested PCR protocol was used for detection of the P2X₂ receptor transcript. PCR products were excised from the gel and reamplified for 28 cycles using the same antisense primer and a sense primer corresponding to position 1059-1078. PCR products were sequenced at the University of Utah Sequencing Center.

(2) Immunohistochemistry.

20 All animal procedures were approved by the University of Utah Institutional Animal Care and Use Committee, and all applicable guidelines from the National Institutes of Health Guide for Care and Use of Laboratory Animals were followed. Olfactory epithelium from post-natal day 4 mice was dissected and post-fixed for 2 hours and then cryoprotected, oriented in Tissue Tek OCT and quickly frozen. Cryostat sections (14 μ m) were permeabilized with 0.3% triton X-100 in PBS, blocked with 10% normal donkey serum.
25 Double-labeling was performed by simultaneously incubating slices in goat anti-OMP (1:10K; generous gift from F. Margolis) and either rabbit anti- P2X₁, P2X₂, P2X₄, P2Y₂ (all 1:100; Alomone Labs, Jerusalem, Israel), or P2X₂ (3 mg/ml; Oncogene Research Products, Boston, MA) overnight followed by a 30 min. incubation in TRITC-conjugated donkey anti-goat immunoglobulin secondary antibody plus FITC-conjugated donkey anti-rabbit immunoglobulin secondary antibody (1:100) (both from Jackson ImmunoResearch Labs,
30 West Grove, PA). For pre-absorption controls, P2 antibodies were incubated with a saturating amount of peptide immunogen (10X) for 1-2 hours and visualized as above.

(3) Olfactory Epithelium Slices and Primary Cultures.

To prepare olfactory epithelium slices, neonatal mice (postnatal day 0-6) were quickly decapitated, and the skin and lower jaw were removed. Tissue was mounted in ice cold Ringers onto a vibratome-cutting
35 block and 300 μ m slices were made. Primary cultures of mouse ORNs were made using the same protocol and culture conditions as described for rat olfactory receptor neurons (Vargas and Lucero, 1999a). Briefly, tissue was placed in divalent cation-free Ringers containing 10 mg/ml bovine serum albumin, 1 mg/ml deoxyribonuclease II and 44 U/ml dispase, incubated at 37°C for 45 min. The tissue was washed, triturated,

and filtered through a 53 mm mesh and 200 ml cells were plated onto concanavalin A-coated coverslips and allowed to settle for 20 min. An additional 1.5 ml of culture medium was added (DMEM supplemented with 100 mM ascorbic acid, 1X insulin-transferrin-seleniumX (GIBCO BRL), 2 mM glutamine, 100 U/ml penicillin G and 100 mg/ml streptomycin).

5 (4) Electrophysiology.

The nystatin perforated-patch technique (Horn and Marty, 1988) was used to examine cells under voltage-clamp. Electrodes (2–5 M Ω) were filled with TMA-oxide internal solution (in mM: 62.5 TMA oxide, 62.5 KH₂PO₄, 15 KCl, 5 MgCl₂, 11 EGTA, 10 HEPES, 1 glutathione, 5 TEA, 0.03% pluronic acid F-127, 0.3% DMSO, 150 mg/ml nystatin, pH 7.2, 330 mOsm).

10 Electro-olfactogram and on-cell recordings: Slices of P0–P6 mouse OE were prepared as described above and mounted in a perfusion chamber with a bath flow of 3 ml/min. Test chemicals were introduced using a rotary injection valve (Rheodyne, Cotati, CA). The electro-olfactogram (EOG) recording electrode (3 M NaCl in 1% agar; tip diameter, 5–10 μ m) was positioned along the dorsal portion of the nasal septum. The differential electrode (identical to the recording electrode) was positioned over skull cartilage and an
15 Ag²/AgCl₂ ground electrode was connected to the bath solution via a 3 M KCl agar bridge. Responses to test agents were amplified (5000 X gain) and filtered (2 kHz) by a low-noise differential DC amplifier. Data was digitized (100 Hz) using Axoscope 8.0 software (Axon Instruments).

For the noninvasive on-cell recordings (Chiu et al., 1997), the same electronics were used as described for nystatin-patch experiments. The recording electrode (5–8 M Ω resistance) contained Ringer's
20 solution. Test solutions were selected using a rotary valve and delivered for 30 sec using gravity flow. The time course of solution delivery was determined by placing an electrode in a slice and switching from Ringer's solution to distilled water. There was a 3 sec delay to initial electrical response, which peaked at 10 sec. The shaded region in Figure 8 shows the 30 sec window of when the valve was switched on and off. During a recording, the electrode was lowered into the dorsal septal region of the slice and a seal (0.5–1 Gohms) was
25 made in voltage-clamp before switching to current-clamp with zero applied current. Only cells with a stable baseline were used. There was a 7 min wash between each test application. Experiments were conducted on 65 cells in 42 slices obtained from 14 P0–P6 mice from three litters. Only three cells survived long enough to complete the recovery portion of the >27 min protocol.

(5) Confocal Calcium Imaging.

30 [Ca²⁺]_i was determined using confocal imaging of fluo-4 AM (18 μ M; Molecular Probes) loaded cells and slices. Cells or slices were placed in a laminar flow chamber (Warner Instruments) and perfused continuously with Ringers solution at a flow rate of 1.5–2.0 ml/min. Ringers contained (mM): 140 NaCl, 5 KCl, 1 MgCl₂, 2 CaCl₂, 10 HEPES, 10 glucose, 500 probenidol and 400 nM tetrodotoxin. Test solutions were applied using bath exchange and a small volume loop injector (200 μ l). A Zeiss LSM 510 confocal laser
35 scanning system was used for data collection and analysis. Time series experiments were performed collecting 1400 x 700 pixel images at 0.2–0.4 Hz. The fluorometric signals obtained are expressed as relative

fluorescence change, $\Delta F/F = (F - F_0)/F_0$, where F_0 is the basal fluorescence level. Increases in F greater than 10% above baseline noise were considered responses.

2. Example 2 Purinergic Receptor Antagonists Potentiate Odor Sensitivity

If the predominate role of endogenous ATP is to reduce odor sensitivity then addition of purinergic receptor antagonists should potentiate odor responses. Control experiments in which we applied odors at 5 - 8 min intervals revealed a small linear increase in the peak amplitude of the odor-induced Ca^{2+} transient (Fig. 12A & C). A linear regression between the first and last odor application was performed and the predicted amplitude of the middle response was calculated. Based on linear regressions, the actual amplitude of the middle odor application was not significantly different from the predicted amplitude, both in the single cell shown in Fig. 12A and in the average of 30 cells in Fig. 12C ($n = 30$; $-3 \pm 4\%$ difference; paired Student's t -test, $p = 0.47$). In contrast, when the middle odor application was preceded by and concomitant with perfusion of purinergic receptor antagonists (100 μM suramin + 25 μM PPADS), a significant increase in the Ca^{2+} transient amplitude such that the mean observed response was $14 \pm 5\%$ larger than the predicted ($n = 22$; paired Student's t -test, $p = 0.024$). The differences between predicted and observed were statistically different when the control group was compared to the purinergic receptor agonist-treated group (independent Student's t -test, $p = 0.012$). Application of purinergic receptor antagonists alone did not evoke calcium transients (Fig. 12B second trace). The elevated odor-evoked calcium transients would be expected if basal extracellular ATP were habitually reducing ORN sensitivity. Thus, the data shows that both endogenous and exogenous ATP reduces the amplitude of odor-evoked calcium transients through purinergic receptors, suggesting that ATP modulates ORN sensitivity.

3. Example 3 ATP Reduces Cyclic Nucleotide-Induced Electrical Responses

Odor activation of G-protein-coupled receptors results in increased cAMP production, opening of cyclic nucleotide-gated channels, influx of Ca^{2+} and Na^+ , depolarization of the membrane, and activation of voltage- and Ca^{2+} -gated ion channels (Schild and Restrepo, 1998). Based on calcium imaging data, purinergics can reduce the odor-evoked electrical activity of ORNs. Recording odor-evoked membrane responses from single ORNs has a low probability of success because each ORN expresses only one or a few odorant receptors (Buck and Axel, 1991). Thus, a mixture of cyclic nucleotide modulators were used to record membrane responses: IBMX (100 μM), a phosphodiesterase inhibitor that prevents the breakdown of cAMP, CPT-cAMP (50 μM), and 8-Br-cGMP (50 μM), both membrane-permeant analogs of cAMP and cGMP, respectively. This cyclic nucleotide mixture was tested initially to verify that it evoked membrane potential changes in the OE slice preparation. The EOG measures field potential changes across the OE after stimulation. Similar EOG responses were obtained from both odor (10 μM) and the cyclic nucleotide mixture (Fig. 13A), validating the replacement of odor with the mixture in subsequent recordings. Next on-cell current-clamp recordings of ORNs were performed from neonatal mouse slices. The cyclic nucleotide mixture, the mixture + ATP, a second application of the mixture, and ATP was sequentially superfused onto a slice preparation and membrane potential changes were measured (Fig. 13B). The coapplication of ATP and the cyclic nucleotide mixture suppressed the cyclic nucleotide-induced electrical responses. The membrane response from each ORN was integrated from baseline and normalized to the initial cyclic nucleotide mixture

response. The presence of ATP reduced the electrical activity of the ORN by $67 \pm 2\%$ (Fig. 13C) ($n = 3$ cells; $p < 0.01$, Newman-Keuls *post hoc* test). These data show that ATP modulates odor sensitivity in mammalian olfactory neurons.

5

G. Sequences

1. SEQ ID NO:1 The following is the sequence for H.sapiens mRNA for ATP receptor P2X1 (accession number X83688). Other sequences have been published for P2X1 receptors from rat vas deferens (accession number X80477) and mouse urinary bladder (accession number X84896).

```

10      1 gaattcggct gatcccgcg gagggtgctag caggagctgg cagcatgggc tccccagggg
      61 ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact
      121 ggccgggtggg cgccctgcag aggtgctgc agtttgggat cgtggtctat gtggtagggt
      181 gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaacccag tttccatca
      241 tcacaaaact caaaggggtt tccgtcactc agatcaagga gcttggaaac cggctgtggg
      15  301 atgtggccga cttcgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact
      361 tccttgtgac gccagcccaa gttcagggca gatgccaga gcaccctcc gtcccactgg
      421 ctaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacggtg
      481 taaaaacagg ccagtgtgtg gtgttcaatg ggacccacag gacctgtgag atctggagtt
      541 ggtgccaggt ggagagtggc gttgtgccct cgaggccccct gctggcccag gcccagaact
      20  601 tcacactgtt catcaaaaac acagtacact tcagcaagtt caacttctct aagtccaatg
      661 ccttggagac ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc
      721 cctactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg
      781 acctggcggt gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca
      841 ccggggactc tggctgctgg cctcactact ccttccagct gcaggagaag agctacaact
      25  901 tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgcacc ctgctcaagc
      961 tctatggaat ccgcttcgac atcctcgtca ccgggcaggc agggaaagtc gggctcatcc
      1021 ccacggccgt cacactgggc accggggcag ctgggtggg cgtggtcacc tttttctgtg
      1081 acctgctact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg
      1141 aggccaaagg cccgaaagca accgccaact ctgtgtggag ggagctggcc tttgcatccc
      30  1201 aagcccagct ggccgagtgc ctcagacgga gctcagcacc tgcaccacg gccactgctg
      1261 ctgggagtag gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa
      1321 ccatttcggg gagcctgtag ccgtttccct gctggttgag aagagagagg ggctgggcaa
      1381 ggaaggaccc ctgcctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat
      1441 caagccgaat tc

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35

2. SEQ ID NO:2 The following is the sequence for H.sapiens protein for ATP receptor P2X1 (accession number X83688).

```

      MARRFQEELAAFLFEYDTPRMVLVRNKKVGVIFRLIQLVVLVYV
      IGWVFLYEKGYQTSSGLISSVSVKLKGLAVTQLPGLGPQVWDVADYVFPAQGDNSFVV
      40  MTNFIIVTPKQTQGYCAEHPEGGICKEDSGCTPGKAKRKAQGIRTGKCVAFNDTVKTCE
      IFGWCPVEVDDDIIPALLREAENFTLFIKNSISFPRFKVNRRLVEEVNAAHMTCL
      FHKTLHPLCPVQLGYVVQESQNFSTLAEGGVGITIDWHCDLDWVHRHCRPIYEF
      HGLYEEKNLSPGFNFRFARHFVENGTNYRHLFKVFGIRFDILVDGKAGKFDIIPMTT
      IGSIGIFGVATVLCDLLLHILPKRHYYKQKKFYAEDMGPGAERDLAATSSTLGL
      45  QENMRTS

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3. SEQ ID NO:3 The following sequence for the P2X2 receptor is derived from rat PC12 cells (accession number U14414). Other sequences have been published for P2X2 receptors from rat cerebellum (accession number Y09910)

```

50      1 gaattcggct gatcccgcg gagggtgctag caggagctgg cagcatgggc tccccagggg
      61 ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact
      121 ggccgggtggg cgccctgcag aggtgctgc agtttgggat cgtggtctat gtggtagggt
      181 gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaacccag tttccatca
      241 tcacaaaact caaaggggtt tccgtcactc agatcaagga gcttggaaac cggctgtggg
      55  301 atgtggccga cttcgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact
      361 tccttgtgac gccagcccaa gttcagggca gatgccaga gcaccctcc gtcccactgg

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421 ctaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacgggtg
 481 taaaaacagg ccagtgtgtg gtgttcaatg ggacccacag gacctgtgag atctggagtt
 541 ggtgcccagt ggagagtggc gttgtgccct cgaggcccct gctggcccag gcccagaact
 601 tcacactgtt catcaaaaac acagtcacct tcagcaagtt caacttctct aagtccaatg
 5 661 ccttggagac ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc
 721 cctactgtcc cgtgttcgcg attggggacc tcgtggccaa ggctggaggg accttcgagg
 781 acctggcggt gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca
 841 ccggggactc tggctgctgg cctcactact ccttcagct gcaggagaag agctacaact
 901 tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgcacc ctgctcaagc
 10 961 tctatggaat ccgcttcgac atcctcgtca ccgggcaggc agggaagttc gggtcatcc
 1021 ccacggccgt cacactgggc accggggcag cttggctggg cgtggtcacc ttttctgtg
 1081 acctgctact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg
 1141 aggccaaagg cccgaaagca accgccaact ctgtgtggag ggagctggcc tttgcatccc
 1201 aagcccagct ggccgagtgc ctcagacgga gctcagcacc tgcacccacg gccactgctg
 15 1261 ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa
 1321 ccattccgg gagcctgtag ccgtttccct gctggttag aagagagagg ggctgggcaa
 1381 ggaaggaccc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat
 1441 caagccgaat tc

20 4. SEQ ID NO:4 The following sequence for the P2X2 receptor is derived from rat
 PC12 cells (accession number U14414) protein sequence.

25 MVRRLARGCWSAFWDYETPKVIVVRNRLGFVHRMVQLLILLYF
 VWYVFIVQKSYQDSETPPESSIIITKVGITMSEDKVWDVEEYVKPPEGGSVVSIITRI
 EVTPSQTGLGTCPESMRVHSSTCHSDDDCIAGQLDMQNGIRTGHCVPYYHGDSKTCEV
 SAWCPVEDGTSNDHFLGKMAPNFTILIKNSIHYPKFKFSKGNIASQKSDYLKHCTFDQ
 DSDPYCPIFRLGFIVEKAGENFTELAHKGGVIGVIINWNCDDLSESECNPKYSFRRL
 DPKYDPASSGYNFRFAKYKINGTTTTRTLIKAYGIRIDVIVHGQAGKFSLIPTIINL
 ATALTSIGVGSFLCDWILLTFMNKNKLYSHKKFKDVRTPKHPSSRWPVTIALVLGQIP
 30 PPPSHYSQDQPPSPSGEGPTLGEGAEPLAVQSPRPCSI SALTEQVVDTLGQHMQR
 PPVPEPSQQDSTSTDPKGLAQL

5. SEQ ID NO:5 The following sequence for the P2X3 receptor is derived from
 H.sapiens (accession number Y07683). Other sequences have been published for P2X3
 receptors from rat dorsal root ganglion (accession number X91167 and X90651).

35 1 gaattcggct gatcccgccg caggtgctag caggagctgg cagcatgggc tccccagggg
 61 ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact
 121 ggccgggtggg cgccctgcag aggtgctgct agtttgggat cgtggtctat gtggtagggt
 181 gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaacccag tttccatca
 40 241 tcaccaaact caaaggggtt tccgtcactc agatcaagga gcttggaac cggtgtggg
 301 atgtggccga cttcgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact
 361 tccttgtgac gccagcccaa gttcaggga gatgccaga gcacccgtcc gtcccactgg
 421 ctaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacgggtg
 481 taaaaacagg ccagtgtgtg gtgttcaatg ggacccacag gacctgtgag atctggagtt
 45 541 ggtgcccagt ggagagtggc gttgtgccct cgaggcccct gctggcccag gcccagaact
 601 tcacactgtt catcaaaaac acagtcacct tcagcaagtt caacttctct aagtccaatg
 661 ccttggagac ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc
 721 cctactgtcc cgtgttcgcg attggggacc tcgtggccaa ggctggaggg accttcgagg
 781 acctggcggt gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca
 841 ccggggactc tggctgctgg cctcactact ccttcagct gcaggagaag agctacaact
 50 901 tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgcacc ctgctcaagc
 961 tctatggaat ccgcttcgac atcctcgtca ccgggcaggc agggaagttc gggtcatcc
 1021 ccacggccgt cacactgggc accggggcag cttggctggg cgtggtcacc ttttctgtg
 1081 acctgctact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg
 55 1141 aggccaaagg cccgaaagca accgccaact ctgtgtggag ggagctggcc tttgcatccc
 1201 aagcccagct ggccgagtgc ctcagacgga gctcagcacc tgcacccacg gccactgctg
 1261 ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa
 1321 ccattccgg gagcctgtag ccgtttccct gctggttag aagagagagg ggctgggcaa
 1381 ggaaggaccc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat
 60 1441 caagccgaat tc

6. SEQ ID NO:6 The following sequence for the P2X3 receptor is derived from
H.sapiens (accession number Y07683) protein sequence.

MNCISDFFTYETTKSVVVKSWTIGIINRVVQLLIISYFVGWVFL
 HEKAYQVRDTAIESSVVTKVKGSGLYANRVMDVSDYVTPPQGTSVFVIITKMIVTENQ
 5 MQGFCEPESEEKYRCVSDSQCGPEPLPGGGILTGRVCVNYSSVLRTCEIQGWCPTVEDTV
 ETPIMMEAENFTIFIKNSIRFPLNFKEGNLLPNLTARDMKTCTRFHPDKDPFCPILRV
 GDVVKFAGQDFAKLARTGGVLGIKIGWVCDLDAWDQCIPKYSFTRLDVSEKSSVSP
 GYNFRFAKYKYMENGSEYRLLKAFGIRFDVLVYGNAGKFNIPTIISVAAFTSVGV
 10 GTVLCDIILLNFKGADQYKAKKFEVNETTLKIAALTNPVYPSDQTAEKQSTDSGA
 FSIQH

7. SEQ ID NO:7 The following sequence for the P2X4 receptor is derived from
H.sapiens (accession number Y07684). Other sequences have been published for P2X4
receptors from rat brain (accession number X93565, U32497, X91200 and X87763) and
rat pancreatic islet (accession number U47031).

1 gaattcggt gatcccgcg caggtgctag caggagctgg cagcatgggc tccccagggg
 61 ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact
 121 ggcgggtggg cgccctgcag aggctgctgc agtttgggat cgtggtctat gtggtagggt
 181 ggcctctcct cgccaaaaa ggctaccagg agcgggacct ggaacccag ttttccatca
 20 241 tcacaaact caaagggtt tccgtcactc agatcaagga gcttggaac cggctgtggg
 301 atgtggccga ctctgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact
 361 tcttgtgac gccagcccaa gttcaggga gatgcccaga gcaccgctc gtcccactgg
 421 ctaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacggtg
 25 481 taaaaacagg ccagtgtgtg gtgttcaatg ggacccacag gacctgtgag atctggagtt
 541 ggtgcccagt ggagagtggc gttgtgccct cgaggcccct gctggcccag gcccagaact
 601 tcacactgtt catcaaaaac acagtacact tcagcaagtt caacttctct aagtccaatg
 661 ccttgagac ctgggacccc acctatttta agcactgccc ctatgaacca caattcagcc
 721 ctaactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg
 30 781 acctggcgtt gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca
 841 ccggggactc tggctgctgg cctcactact ccttccagct gcaggagaag agctacaact
 901 tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgcacc ctgctcaagc
 961 tctatggaat ccgcttcgac atcctcgtca ccgggcaggc agggagttc gggctcatcc
 1021 ccacggccgt cacactggc accggggcag cttggctggg cgtggtcacc ttttctgtg
 35 1081 acctgctact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg
 1141 aggccaaggc ccgaaagca accgcaact ctgtgtggag ggagctggcc tttgcatccc
 1201 aagcccact ggccagtg ctcagacgga gctcagcacc tgcacccacg gccactgctg
 1261 ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cactgccaa
 1321 ccattccgg gagcctgtag ccgttccct gctggttgag aagagagagg ggctgggcaa
 40 1381 ggaaggacc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat
 1441 caagccgaat tc

8. SEQ ID NO:8 The following sequence for the P2X4 receptor is derived from
H.sapiens (accession number Y07684) Protein sequence.

45 MAGCCSALAAFLFEYDTPRIVLIRSRKVLNRAVQLLILAYVI
 GWVFWKEGYQETDSVSVVTKVKGVAVTNTSKLGFRIWDVADYVIPAQEENSLFVM
 TNVILTMNQTLQCLPEIPDATTVCKSDASCTAGSAGTHNGVSTGRVAFNGSVKTCE
 VAAWCPVEDDTHVPQPAFLKAAENFTLLVKNNIWPKNFNSKRNLPNITTYLKSCI
 YDAKTDPFCPIFRLGKIVENAGHSFQDMAVEGGIMGIQVNWDCNLDRAASLCLPRYSF
 50 RRLDTRDVEHNVS PGYNFRFAKYRDLAGNEQRTLIKAYGIRFDIIVFGKAGKFDIIP
 TMINIGSGLALLGMATVLCDIIVLYCMKKRLYYREKKYKYVEDYEQGLASELDQ

9. SEQ ID NO:9 The following sequence for the P2X5 receptor is derived from
H.sapiens (accession number AF016709). Other sequences have been published for
P2X5 receptors from rat brain (accession number X92069) and rat heart (accession
number X97328).

1 ggcacgaggg tccgcaagcc cggctgagag cgcgccatgg ggcaggcggg ctgcaagggg

5 61 ctctgcctgt cgtgtgtcga ctacaagacc gagaagtatg tcatcgccaa gaacaagaag
121 gtgggcctgc tgtaccggct gctgcaggcc tccatcctgg cgtacctggc cgtatgggtg
181 ttcctgataa agaaggggta ccaagacgtc gacacctccc tgcagagtgc tgtcatcacc
241 aaagtcaagg gcgtggcctt caccaacacc tcggatcctt ggagcggat ctgggatgtc
301 gccgactacg tcattccagc ccaggggagag aacgtctttt ttgtgggtcac caacctgatt
361 gtgaccccca accagcggcga gaacgtctgt gctgagaatg aaggcattcc tgatggcgcg
421 tgctccaagg acagcgactg ccacgctggg gaagcgggta cagctggaaa cggagtgaag
481 accggccgct gcctgcggag agggaaacttg gccaggggca cctgtgagat ctttgccctg
541 tgcccgttg agacaagctc caggccggag gagccattcc tgaaggaggc cgaagacttc
10 601 accattttca taaagaacca catcgtttc cccaaattca acttctccaa aaacaatgtg
661 atggacgtca aggacagatc tttcctgaaa tcatgccact ttggcccaaa gaaccactac
721 tgccccatct tccgactggg ctccatcgtc cgctgggccc ggagcgactt ccaggatata
781 gccctgcgag gtggcgtgat aggaattaat attgaatgga actgtgatct tgataaagct
841 gcctctgagt gccaccctca ctattctttt agcctgtctg acaataaact ttcaaagtct
15 901 gtctcctccg ggtacaactt cagatttgcc agatattacc gagacgcagc cgggggtggag
961 ttccgcaccc tgatgaaagc ctacgggatc cgctttgacg tgatggtgaa cggcaagggt
1021 gctttcttct ggcacctggg actcatctac ctcacaaaa agagagagtt ttaccgtgac
1081 aagaagtacg aggaagtga gggcctagaa gacagttccc aggaggccga ggacgaggca
1141 tcggggctgg ggctatctga gcagctcaca tctgggccag ggctgctggg gatgccggag
20 1201 cagcaggagc tgcaggagcc acccgaggcg aagcgtggaa gcagcagtc gaaggggaac
1261 ggatctgtgt gccacagct cctggagccc cacaggagca cgtgaattgc ctctgcttac
1321 gttcaggccc tgtcctaaac ccagccgtct agcaccagc gatcccatgc ctttgggaat
1381 ccaggatgc tgcacaacgg gaaatttgta cattgggtgc tatcaatgcc acatcacagg
1441 gaccagccat cacagagcaa agtgacctcc acgtctgatg ctggggatcat caggacggac
25 1501 ccatcatggc tgtctttttg ccccccccc tgccgtcagt tcttcttct tccgtggctg
1561 gcttcccga ctagggaacg ggttgtaaat ggggaacatg acttccttcc ggagtccttg
1621 agcacctcag ctaaggaccg cagtgccttg tagagtctct agattacctc actgggaata
1681 gcattgtgct tgtccggaaa agggctccat ttggttccag cccactcccc tctgcaagtg
1741 ccacagcttc cctcagagca tactctccag tggatccaag tactctctct cctaaagaca
30 1801 ccaccttctt gccagctgtt tgcccttagg ccagtagaca gaattaaagt gggggagatg
1861 gcagacgctt tctgggacct gcccaagata tgtattctct gacactctta tttggtcata
1921 aaacaataaa tgggtgtcaat ttcaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa

10. SEQ ID NO:10 The following sequence for the P2X5 receptor is derived from

35 *H.sapiens* (accession number AF016709) protein sequence.

MGQAGCKGLCLSLFDYKTEKYVIAKNKKVGLLYRLLQASILAYL
VWVFLIKKGYQDVDTSLQSAVITKVKGVAFTNTSDLQRIWDVADYVIPAQGENVFF
VVTNLIVTPNQRQNVCAENEGIPDGACSKSDSCHAGEAVTAGNGVKTGRCLRRGNLAR
40 GTCEIFAWCPLETSSRPEEPFLKEAEDFTIFIKNHIRFPKFNFSKNNVMDVKDRSFLK
SCHFGPKNHYPICFRLGSIWRWAGSDFQDIALRGGVIGINIEWNCDLDKAASECHPHY
SFSRLDNKLSKSVSSGYNFRFARYYRDAAGVEFRTLMKAYGIRFDMVNGKGAFFCDL
VLIYLIKREBYRDKKYEBVRGLEDSQEADEASGLGLSEQLTSGPGLLGMPQEQL
QEPPEAKRGSSSQKNGSVCPQLLEPHRST

11. SEQ ID NO:11. The following sequence for the P2X6 receptor is derived from

H.sapiens (accession number AF065385). Other sequences have been published for
P2X6 receptors from rat brain (accession numbers X92070 and X97376).

50 1 gaattcggct gatcccgagg cagggtctag caggagctgg cagcatgggc tccccagggg
61 ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact
121 ggcgggtggg cgccctgcag aggctgctgc agtttgggat cgtggtctat gtggtagggt
181 gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaacccag ttttccatca
241 tcaccaaact caaagggggt tccgtcactc agatcaagga gcttggaac cggctgtggg
301 atgtggccga cttcgtgaag ccacctcagg gagagaacgt gttctctctg gtgaccaact
55 361 tccttgtgac gccagcccaa gtacggggca gatgcccaga gcacctgtcc gtcccactgg
421 ctaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacgggtg
481 taaaaacagg ccagtgtgtg gtgttcaatg ggacccacag gacctgtgag atctggagtt
541 ggtgccccag ggagagtggc gttgtgccct cgaggccccct gctggccccg gccagaaact
60 601 tcacactgtt catcaaaaac acagtcacct tcagcaagtt caacttctct aagtccaatg
661 ccttgagagc ctgggacccc acctatttta agcactgccc ctatgaacca caattcagcc
721 cctactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg
781 acctggcgct gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca
841 ccggggactc tggctgctgg cctcactact ccttccagct gcaggagaag agctacaact
901 tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgacc ctgctcaagc

961 tctatggaat ccgcttcgac atcctcgtca cggggcaggc aggggaagttc gggctcatcc
 1021 ccacggccgt cacactgggc accggggcag cttggctggg cgtggtcacc ttttctgtg
 1081 acctgtact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg
 1141 agggcaaggc cccgaaagca accgccaact ctgtgtggag ggagctggcc tttgcatccc
 5 1201 aagcccgaact ggccgagtgc ctgagacgga gctcagcacc tgcacccacg gccactgctg
 1261 ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa
 1321 cccattccgg gagcctgtag ccgtttccct gctggttgag aagagagagg ggctgggcaa
 1381 ggaaggacc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat
 1441 caagccgaat tc

10

12. SEQ ID NO:12. The following sequence for the P2X6 receptor is derived from

H.sapiens (accession number AF065385) protein sequence.

MGSPGATTGWGLLDYKTEKYVMTRNWRVGALQRLQLQFGIVVYVV
 15 GWALLAKKGYQERDLEPQFSIITKLKGVSVTQIKELGNRLWDVADFVKPPQGENVFFL
 VTNFLVTPAQVQGRCPVPLANCWVDEDCPEGEGGTHSHGVKTGQCVVFNGTHRT
 CEIWSWCPVESGVVPSRPLLAQAQNFILFIKNTVTFSKFNFSKSNALETWDPYFKHC
 RYEPQFSPYCPVFRIGDLVAKAGGTFEDLALLGSSVGIRVHWDCLDLDGSGCWPHYS
 20 FOLQEKSYNFRATATHWQPGVEARTLLKLYGIRFDILVTGQAGKFLIPTAVTLGTG
 AAWLGVVTFCDLLLYVDREAHFYWRKYEEAKAPKATANSVWRELAFASQARLAEC
 LRRSSAPAPTATAAGSQTQTPGWPCPSSDTHLPHSGSL

13. SEQ ID NO:13 The following sequence for the P2X7 receptor is derived from

H.sapiens brain (accession number Y09561). Please note that other sequences have been

published for P2X7 receptors from rat brain (accession numbers X95882)

25

1 aaaacgcagg gagggaggct gtcaccatgc cggcctgctg cagctgcagt gatgttttcc
 61 agtatgagac gaacaaagtc actcggatcc agagcatgaa ttatggcacc attaatgggt
 121 tcttccacgt gatcatcttt tctacgctt gctttgctct ggtgagtgac aagctgtacc
 181 agcggaaaga gctgtcatc agttctgtgc acaccaaggt gaaggggata gcagagggtga
 241 aagaggagat cgtggagaat ggagtgaaga agttggtgca cagtgtcttt gacaccgcag
 301 actacacctt cctttgagc gggaactcct tcttctgtat gacaaaactt ctcaaaacag
 361 aaggccaaga gcagcgggtg tgtcccgagt atcccaccg caggacgctc tgttctctg
 421 accgagggtg taaaaaggga tggatggacc cgcagagcaa aggaattcag accggaaggt
 481 gtgtagtga tgaagggaac cagaagacct gtgaagtctc tgcctgggtg cccatcgagg
 541 cagtgggaaga gggcccccgg cctgctctct tgaacagtgc cgaaaacttc actgtgctca
 601 tcaagaacaa tatcgacttc cccggccaca actacaccac gagaacatc ctgccagggt
 661 taaacatcac ttgtaccttc cacaagactc agaatccaca gtgtccattc ttccgactag
 721 gagacatctt ccgagaaaca ggcgataatt ttccagatgt ggcaattcag ggcggaataa
 781 tgggcattga gatctactgg gactgaacc tagaccgtt gttccatcac tgccatccca
 841 aatacagttt ccgtcgctt gacgacaaga ccaccaacgt gtccttgtac cctggctaca
 901 acttcagata cgccaagtag tacaaggaaa acaatgttga gaaacggact ctgataaaag
 961 tcttcgggat ccgttttgac atcctgggtt ttggcaccgg agggaaaattt gacattatcc
 1021 agctgggtgt gtacatcggc tcaaccctct cctacttcgg tctggccgct gtgttcatcg
 1081 acttcctcat cgacacttac tccagtaact gctgtcgtc ccatatttat ccctgggtga
 1141 agtgctgtca gccctgtgtg gtcaacgaat actactacag gaagaagtgc gactgcattg
 1201 tggagccaaa gccgacatta aagtatgtgt cctttgtgga tgaatccac attaggatgg
 1261 tgaaccagca gctactaggg agaagtctgc aagatgtcaa gggccaagaa gtcccaagac
 1321 ctgcgatgga ctccacagat ttgtccaggc tgcccctggc cctccatgac acacccccga
 1381 ttcctggaca accagaggag atacagctgc ttagaaagga ggcgactcct agatccaggg
 1441 atagccccgt ctggtgccag tgtggaagct gcctccatc tcaactccct gagagccaca
 1501 ggtgcctgga ggagctgtgc tgccggaaaa agccgggggc cctcatcacc acctcagagc
 1561 tgttcaggaa gctggtcctg tccagacacg tctgtcagtt cctcctgtc taccaggagc
 1621 ccttgctggc gctggatgtg gattccacca acagccggct gcggcactgt gcctacaggt
 1681 gctacgccac ctggcgcttc ggctcccagg acatggctga ctttgccatc ctgcccagct
 1741 gctgccgctg gaggatccgg aaagagtctt cgaagagtga agggcagtag agtggttca
 1801 agagtcctta ctgaagccag gcaccgtggc tcacgtctgt aatccacct ttt

14. SEQ ID NO:14 The following sequence for the P2X7 receptor is derived from

H.sapiens brain (accession number Y09561) protein sequence

60

MPACCSVDVFQYETNKVTRIQSMNYGTIKWFFHVIIIFSVCFA

LVSDKLYQRKEPVISSVHTKVKGIAEVKEEIVENGVKLVHSVFDTADYTFPLQGNSF
 FVMTNFLKTEGQEQRLCPEYPTRRITLCSSDRGCKKGWMDPQSKGIQTGRCVVHEGNQK
 TCEVSAWCP IEAVEEAPRALLNSAENFTVLIKNNIDFPGHNYTTRNIPGLNITCTF
 5 HKTQNPQCP IFR LGDI FRETGDNFSDVAIQGGIMGIEIYWDNLDLRFHCHPKYSFR
 RLDDKTTNVSFLYPGYNFRYAKYKNNVKEKRTLIKVFGRFDILVFGTGGKFDIIQLV
 VYIGSTLSYFGLAAVFIDFLIDTYSSNCCRSHIYPWCKCCQPCVVNEYYYRKCESIV
 BPKPTILKYVSFVDESHIRMVNQQLGRSLQDVKGQEVPRPAMDFTDL SRLPLALHDTF
 PIPGQPEEIQLLRKEATPRSRDSPVWCQCGSCLPSQLPESHRCLEELCCRKKPGACIT
 10 TSELFKRKLVL SRHVLQFLLLYQEPL LALD VDSTNSRLRHCA YRCYATWRFGS QDMA DF
 AILPSCCRWRIRKEFPKSEGYSGFKSPY

15 **15. SEQ ID NO:15** The following sequence for the P2Y1 receptor is derived from
H.sapiens (accession number S81950). Other sequences have been published for P2Y1
 receptors from human placenta (accession number Z49205), HEL cells (accession
 number U42030), bovine endothelium (accession number X87628), rat cells (accession
 numbers U22830 and U22829), turkey brain (accession number U09842) and chicken
 brain (accession number X73268).

1 ggatccaggtt cgcctgctcc cttccgctcg ctggcttttc cgatgcttgc tgcgcccctg
 20 61 gccgcgctg cctctcgcgc gccctcctacc cctcggagcc gccgcctaag tcgaggagga
 121 gagaatgacc gaggtgctgt ggcgggctgt ccccaacggg acggacgctg ccttcctggc
 181 cggtcggggt tcgtcctggg ggaacagcac ggtcgctcc actgcgcgcg tctcctcgtc
 241 gttcaaatgc gccttgacca agacgggctt ccagttttac tacctgccgg ctgtctacat
 301 cttgggtattc atcatcggct tcctgggcaa cagcgtggcc atctggatgt tcgtcttcca
 25 361 catgaagccc tggagcggca tctccgtgta catgttcaat ttggctctgg ccgacttctt
 421 gtacgtgctg actctgccag ccctgatctt ctactacttc aataaaacag actggatctt
 481 cggggatgcc atgtgtaaac tgcagagggt catctttcat gtgaacctct atggcagcat
 541 cttgtttctg acatgcatca gtgcccaccg gtacagcggt gtggtgtacc ccctcaagtc
 601 cctgggcccg ctcaaaaaga agaatgcgat ctgtatcagc gtgctggtgt ggctcattgt
 30 661 ggtgggtggcg atctccccc tctcttctta ctcagggtacc ggggtccgca aaaacaaaac
 721 catcacctgt tacgacacca cctcagacga gtacctgcga agttatttca tctacagcat
 781 gtgcacgacc gtggccatgt tctgtgtccc cttggtgctg attctgggct gttacggatt
 841 aattgtgaga gctttgattt acaaagatct ggacaactct cctctgagga gaaaatcgat
 901 ttacctggta atcattgtac tgactgtttt tgctgtgtct tacatccctt tccatgtgat
 35 961 gaaaacgatg aacttgaggg cccggcttga ttttcagacc ccagcaatgt gtgctttcaa
 1021 tgacagggtt tatgccacgt atcagggtgac aagagggtcta gcaagtctca acagtgtgtg
 1081 ggaccccatc ctctatttct tggcgggaga tactttcaga aggagactct cccgagccac
 1141 aaggaaagct tctagaagaa gtgaggcaaa tttgcaatcc aagagtgaag acatgaccct
 1201 caatatatta cctgagttca agcagaatgg agatacaagc ctgtgaaggc acaagaatct
 40 1261 ccaaacacct ctctgttgta atatggtagg atgcttaaca gaatcaagta ct

16. SEQ ID NO:16 The following sequence for the P2Y1 receptor is derived from
H.sapiens (accession number S81950).

45 MTEVLWPAVNGTDA AFLAGPGSSWGNSTVASTAAVSSSFKCAL
 TKTGFQFYFLPAVYILVFIIGFLGNSVAIWMFVFMKPWSGISVYMFNLALADFLYVL
 TLPALIFYFNKTDWIFGDAMCKLQRFIFHVNLYGSILFTLCISAHRYSGVVYPLKSL
 GRLKKNAICISVLVWLIVVVAISPILFYSGTGVRKNKTTTCYDTSDEYLRSYFIYS
 MCTTVAMFCVPLVLILGCYGLIVRALIYKDLDNSPLRRKSIYLVIIIVLTVFAVSYPF
 50 HVMKTMNLRARLDFQTPAMCAFNDRVYATYQVTRGLASLNSCVDPILYFLAGDTFRRR
 LSRATRKASRRSEANLQSKSEDMTLNIPFEFKQNGDTSL

55 **17. SEQ ID NO:17** The following sequence for the P2Y2 receptor is derived from
H.sapiens epithelial cells (accession number U07225). Other sequences have been
 published for P2Y2 receptors from rat alveolar cells (accession number U09402), rat
 pituitary cells (accession number L46865), Wistar Kyoto rat (accession number
 U56839), and mouse neuroblastoma cells (accession number NM_008773).

1 cggcacgagg caccgccgaga ggagaagcgc agcgagtgagg cgagaggagc cccttggtggc

61 agcagcacta cctgcccaga aaaatgctgg aggctgggag tggccccagg cctggggacc
 121 tgttttttcc gttttcccga gagttccctg cagcccgggc cagggtccagg cgtgtgcatt
 181 catgagtgag gaacccgtgc aggcgctgag catcctgacc tggagagcag gggctgggtca
 241 gggcgatggc agcagacctg gggccctgga atgacaccat caatggcacc tgggatgggg
 5 301 atgagctggg ctacagggtg cgcttcaacg aggacttcaa gtacgtgctg ctgcctgtgt
 361 cctacggcgt ggtgtgctg cttgggctgt gtctgaacgc cgtggcgctc tacatcttct
 421 tgtgcccgtc caagacctgg aatgcgtcca ccacatatat gttccacctg gctgtgtctg
 481 atgcactgta tgcggcctcc ctgcgctgct tgggtctatta ctacgcccgc ggcgaccact
 541 gggccttcag caggtgtgct tgcaagctgg tgcgcttctc cttctacacc aacctttact
 10 601 gcagcatcct cttcctcacc tgcacagcgt tgcacgggtg tctgggcgtc ttacgacctc
 661 tgcgctccct gcgctggggc cgggcccgtc acgctcgccg ggtggccggg gccgtgtggg
 721 tgttggtgct ggcctgccag gcccctgtgc tctactttgt caccaccagc gcgcgcgggg
 781 gccgcgtaac ctgccacgac acctcggcac ccgagctctt cagccgcttc gtggcctaca
 841 gctcagtcac gctgggcctg ctcttcgctg tgccttttgc cgtcatcctt gtctgttacg
 15 901 tgctcatggc tcggcgactg ctaaagccag cctacgggac ctcgggcgtc ctcctaggg
 961 ccaagcgcaa gtccgtgctg accatcgccg tgggtgctggc tgtcttcgcc ctctgcttcc
 1021 tgcatttcca cgtcaccgcg acctctact actccttccg ctgctgggac ctcagctgcc
 1081 acaccctcaa cgccatcaac atggcctaca aggttaccgg gccgctggcc agtgctaaca
 1141 gttgccttga ccccgctgctc tacttctctg ctgggcagag gctcgtacgc tttggccgag
 120 1201 atgccaagcc acccactggc ccagccctg ccaccccgcc tcgcccagc cctggcctgc
 1261 gcagatccga cagaactgac atgcagctg taggagatgt gttgggcagc agtgaggact
 1321 tcaggcggac agagtccacg ccggctggta gcgagaacac taaggacatt cggctgtagg
 1381 agcagaacac ttcagcctgt gcaggtttat attgggaagc ttagaggagc caggacttgt
 1441 gcagacgcca cagtctcccc agatatggac catcagtgac tcatgctgga tgaccccatg
 15 1501 ctccgtcatt tgacaggggc tcaggatatt cactctgtgg tccagagtca actgttccca
 1561 taacccttag tcatcgtttg tgtgtataag ttgggggaat taagtttcaa gaaaggcaag
 1621 agtcaaggt caatgacacc cctggcctga ctcccatgca agtagctggc tgtactgcca
 1681 aggtacctag gttggagtcc agcctaatac agtcaaagtg agaaacaggc ccagagagga
 1741 aggtggctta ccaagatcac ataccagagt ctggagctga gctacctggg gtgggggcca
 180 1801 agtcacaggt tggccagaaa acctggtaa gtaatgaggg ctgagtttgc acagtggctc
 1861 ggttgccacg gtggacttag ctctgaggag taccocccgc caagagatg ggagcagtg
 1921 aacatctggg gactaataat atagacccat ctggaggctc ccatgggcta ggagcagtg
 1981 gaggctgtaa cttatactaa aggttgtgtt gcctgctaaa aaaaa

35 18. SEQ ID NO:18 The following sequence for the P2Y2 receptor is derived from

H.sapiens epithelial cells (accession number U07225) protein sequence.

MAADLGPNWDITNGTWDGDELGYRCRFNEDFKYVLLPVSYGVC
 VLGLCLNAVALYIFLCRLKTNASTTYMFHLAVSDALYAASLPPLVYYYARGDHWPF
 TVLCKLVRFLFYTNLYCSILFLTCISVHRCGLVLRPLRLSLRWGRARYARRVAGAVWVL
 40 VLACQAPVLYFVTTTSARGGRVTCHDTSAPLEFSRFVAYSSVMLGLLFAVPFVILVCY
 VLMARRLLKPAYGTSGGLPRAKRKSVRTIAVVLAVFALCFLPFHVTRTLYYSFRSLDL
 SHTLNAINMAYKVTRPLASANSCLDPVLYFLAGQRLVRFARDAKPPTGPSATPARR
 RLGLRRSDRTDMQRIGDVLGSSSEDFRRTSTPAGSENTKDRL

45 19. SEQ ID NO:19 The following sequence for the P2y3 receptor is derived from chick
brain (accession number X98283).

1 ggcgcttcac ccagtaaaga gggaccatga gcatggccaa cttcacgggg gggaggaact
 61 cgtgcacctt ccagtaggaa ttcaagcagg tcctgctgcc cctgggtctac tcagtgggtg
 50 121 tctactggg gctgccactc aatgccgttg tcattgggca gatctggctg gcccgcaagg
 181 cggtgacctg caccaccatc tacatgctga acctggccat ggccgacctg ctttatgtct
 241 gctccctccc tctcctcacc tacaactaca ccagaagga ttactggccc tttggggact
 301 tcacctgcaa attcgtccg ttcagttct acaccaacct gcacggcagc atcctcttcc
 361 tcacctgcat cagcgtccag cgctacatgg ggatctgcca cccctggcc cctgtggcaca
 55 421 aaaagaagg aaagaagctg acgtggctgg tgtgtgctgc cgtgtgggtc atcgtcatcg
 481 ccagtgacct gcccaccttt gtcttcgct ccaccggcac gcagaggaat cgcactgtct
 541 gctatgacct gagccccccg gaccgctcca catcctactt cccctatggc atcacgttga
 601 ccactactgg cttcctgctg ccttcgctg ccatcctggc ctgctactgc agcattggcc
 661 gcactcctgt ccagaaagac gagctgattg gcttggcggt gcacaagaag aaggacaagg
 60 721 ccgtgcgcat gatcatcatc gttgtcatcg tcttctccat cagcttcttc ccttccacc
 781 tcaccaagac catctacctg atcgtccgt cctcagccag cttgccctgc cctacctgc
 841 aggcctttgc cattgcctac aagtgcacgc ggccttttgc cagcatgaac agcgtcctcg
 901 acccatcct cttctacttc acccagcga agtttcgtga gagcaccgc tatctcctgg
 961 acaagatgag ctccaagtgg cggcaagacc actgcatcag ctacggctcc taggtgacg
 65 1021 aggcacctc ggtgtcaccg gggctgggca tggagcaatt tgggttgaag ctgcatggg

1081 cggagatggg gatgagccca gaggctctgcg ggtgccccat ctctggaggt gttggagatt
 1141 agattggatg gggctctggg ccc

- 5 20. SEQ ID NO:20 The following sequence for the P2y3 receptor is derived from chick brain (accession number X98283) protein sequence.

MSMANFTGGRNSCTFHEEFKQVLLPLVYSVVFLGLPLNAVIG
 QIWLARKALTRTTIYMLNLAMADLLVCSLPLLIYNYTQKDYWPFDFCTCKFVRQFY
 10 TNLHGSILFLTCISVQRYMGICHPLASWHKKKGKKLTWLVCAAVWFIVIAQCLPTFVF
 ASTGTQRNRTVCYDLSPDRSTSYFPYGITLTITGFLFPFAAILACYCSMARILCQKD
 ELIGLAVHKKKDKAVRMIIVVIVFSISFFPFHLTKTIYLIVRSSASLPCPTLQAFAI
 AYKCTRPFASMNSVLDPILFYFTQRFRESTRYLLDKMSSKWRQDHCISYGS

- 15 21. SEQ ID NO:21 The following sequence for the P2Y4 receptor is derived from H.sapiens (accession number X91852). Other sequences have been published for P2Y4 receptors from human chromosome X (accession number U40223), and rat heart (accession number Y14705).

20 1 aagggagctt gggtaggggc caggctagcc tgagtgcacc cagatgcgct tctgtcagct
 61 ctccctagt gttcaaccac tgctctccct gctctacttt tttgtctcca gctcagggat
 121 ggggggtggc agggaaatcc tgccaccctc acttctcccc tccccatctc cagggggggc
 181 atggccagta cagagtcctc cctggtgaga tccctaggcc tcagcccagg tcctggcagc
 241 agtgaggtgg agctggactg ttggtttgat gaggatttca agttcatcct gctgcctgtg
 301 agctatgcag ttgtctttgt gctgggcttg ggccttaacg ccccaaccct atggctcttc
 25 361 atcttccgcc tccgaccctg ggatgcaacg gccacctaca tgttccacct ggcattgtca
 421 gacaccttgt atgtgctgtc gctgcccacc ctcatctact attatgcagc ccacaaccac
 481 tggccctttg gcaactgagat ctgcaagttc gtccgcttcc ttttctattg gaacctctac
 541 tgcagtgtcc ttttctctac ctgcatcagc gtgcaccgct acctgggcat ctgccaccca
 601 cttcgggcac tacgctgggg ccgcccctgc ctgcaggcc ttctctgcct ggcagtttgg
 30 661 ttggtcgtag ccggtcgctc cgtgcccac ctgttctttg tcacaaccag caacaaaggg
 721 accaccgtcc tgtgccatga caccactcgg cctgaagagt ttgaccacta tgtgcacttc
 781 agctcggcgg tcatggggtc gctctttggc gtgcccctgc tggtcactct tgtttgctat
 841 ggactcatgg ctgctgcctc gtatcagccc ttgccaggct ctgcacagtc gtcttctcgc
 901 ctccgctctc tccgcaccat agctgtgggt ctgactgtct ttgctgtctg ctctgtgctc
 35 961 ttccacatca cccgcaccat ttactacctg gccaggctgt tgggaagctga ctgccagta
 1021 ctgaacattg tcaacgtggc ctataaagtg actcggcccc tggccagtgc caacagctgc
 1081 ctggatcctg tgctctactt gctcactggg gacaaatata gacgtcagct ccgtcagctc
 1141 tgtgggtggg gcaagcccca gcccgcacg gctgctctt ccctggcact agtgtccctg
 1201 cctgaggata gcagctgcag gtggggcgcc acccccagg acagtagctg ctctactcct
 40 1261 agggcagata gattgtaaca cgggaagccg ggaagtga gaaaagggg tgagtgacgg
 1321 gcagaggtga gggaaaccaa tagtgatac tggttaagggtg cttcttctc ttttccaggc
 1381 tctggagaga agccctcacc ctgaggggtg ccaggggaggc agggatatac

- 45 22. SEQ ID NO:22 The following sequence for the P2Y4 receptor is derived from H.sapiens (accession number X91852) Protein sequence.

MASTESSLLRSLGLSPGPGSSEVELDCWFDEDFKFILLPVSYAV
 VFVLGLGLNAPTLWLFI FRLRPWDATATYMFHLALSDTLVLSLPTLIYYAAHNHWP
 FGTEICKFVRFLFYWNLYCSVFLTCISVHRYLGICHPLRALRWGRPRLAGLLCLAVW
 50 LVVAGCLVPNLFVVTTSNKGTTVLCHDTTRPEEFDHYVHFSSAVMGLLFGVPCLVTLV
 CYGLMARRLYQPLPGSAQSSSRLSLRTIAVVLTVFAVCFVPHITRTIYYLARLLEA
 DCRVLNIVNVVYKVTRPLASANSCLDPVLYLLTGDKYRRLQLCGGKQPRTAASS
 LALVSLPEDSSCRWAATPDSSCSTPRADRL

- 55 23. SEQ ID NO:23 The following sequence for the P2Y6 receptor is derived from H.sapiens placenta (accession number X97058). Other sequences have been published for P2Y6 receptors from human placenta (accession number AF007893), and human activated T-cells (accession number U52464).

1 ctcagtttcc tcattctgctg cctctccaga cttctgccag aacattgcac gcgacagttt
 61 caggcacaga actgactggc agcaggggct gctccacgag tgggaatttg ctccagcact
 121 tcacggactg caagcgagggc acttgctaac tcttgataa caagacctct gccagaagaa
 181 ccatggctttt ggaaggcgga gttcaggctg aggagatggg tgcggtcctc agtgagcccc
 241 tgcctccctg aacataggaa acccacctgg gcagccatgg aatgggacaa tggcacaggc
 301 caggctctgg gcttgccacc caccacctgt gtctaccgag agaacttcaa gcaactgctg
 361 ctgccacctg tgtattcggc ggtgctggcg gctggcctgc cgtgaacat ctgtgtcatt
 421 acccagatct gcacgtcccc cggggccctg acccgcacgg ccgtgtacac cctaaacctt
 481 gctctggctg acctgctata tgcctgctcc ctgcccctgc tcactacaa ctatgccaa
 541 ggtgatcact ggccctttgg cgacttcgac tgcgcctgg tccgcttctt cttctatgcc
 601 aacctgcacg gcagcatcct cttcctcacc tgcacagct tccagcgta cctgggcatc
 661 tggcaccgcg tggccccctg gcacaaacgt gggggccgac gggctgcctg gctagtgtgt
 721 gtacgcgtgt ggctggccgt gacaaacag tgcctgcca cagccatctt cgtgcccaca
 781 ggcatccagc gtaaccgcac tgtctgctat gacctcagc cgcctgcctt gggcaccac
 841 tatatgccct atggcatggc tctcactgtc atcggcttcc tgctgcctt tggctgcccc
 901 ctggcctgct actgtctcct ggccctgcgc ctgtgcccgc aggatggccc ggcagagcct
 961 gtggcccagg agcggcgctg caaggcgcc cgcatggccg tgggtggggc tgcctgcttt
 1021 gccatcagct tctgctctt tcacatcacc aagacagcct acctggcagt gcgctgcagc
 1081 ccggcgctcc cctgcactgt attggaggcc tttgcagcgg cctacaaagg cacgcggccg
 1141 tttgcagtg ccaacagcgt gctggacccc atcctcttct acttcacca gaagaagttc
 1201 cgccggcgac cacatgagct cctacagaaa ctcacagcca aatggcagag gcagggtcgc
 1261 tgagtctctc aggtcctggg cagccttcat atttgccatt gtgtccgggg caccaggagc
 1321 cccaccaacc ccaaaccatg cggagaatta gagttcagct cagctggggc tggagttaag
 1381 atccctcaca ggaccagaa gctcaccaaa aactatttct tcagccctt cctgggccc
 1441 catcctgtgg gcatggagat ggacagacct gggcctggct cttgagaggt cccagtcagc
 1501 catggagagc tggggaaacc acattaaggt gctcacaaa atacagtgtg acgtgtactg
 1561 tcaaaaaaaa a

30 **24. SEQ ID NO:22** The following sequence for the P2Y₆ receptor is derived from
H.sapiens (accession number X91852) Protein sequence.

MEWDNGTGQALGLPPTTCVYRENFKQLLLPPVYSAVLAAGLPLNICVITQICTSRRLTRTA
 VYTLNLALADLLIYACSLPLLIYNYAQGDHWPFGDFACRLVRLFLFYANLHGSILFLTCISFQR
 35 YLGICHPLAPWHKRGRRRAAWLVCVAVWLAVTTQCLPTAIFAATGIQRNRTVCYDLSPPALA
 THYMPYGMALTVIGFLPFAALLACYCLLACRLCRDQGPAPVQAQERRGKAARMAVVVAAAF
 AISFLPFHITKTAYLAVRSTPGVPCTVLEAFAAAYKGRPFASANSVLDPIIFYFTQKKFR
 RPHELLQKLTAKWQRQGR

40 **25. SEQ ID NO:24** The following sequence for the P2Y₁₁ receptor is derived from
human placenta (accession number AF030335). Other sequences have been published
for P2Y₁₁ receptors from human HL-60 cells (accession number AJ298334).

45 1 atggatcgag gtgccaagtc ctgccctgcc aacttcttgg cagctgccga cgacaaactc
 61 agtgggttcc agggggactt cctgtggccc atactggtgg ttgagttcct ggtggccgtg
 121 gccagcaatg gcctggccct gtaccgcttc agcatccgga agcagcggcc atggcaccac
 181 gccgtggtct tctctgtcca gctggcagtc agcgacctgc tctgcgctct gacgtgccc
 241 ccgctggccg cctacctcta tccccccaag cactggcgct atggggaggg cgcgtgccc
 301 ctggagcgct tctcttcac ctgcaacctg ctgggcagcg tcactcttcat cacctgcata
 361 agcctcaacc gctacctggg catcgtgcac ccttctctcg cccgaagcca cctgcgaccc
 421 aagcacgcct gggccgtgag cgtgcccggc tgggtcctgg ccgcccctgt ggccatgccc
 481 aactcagct tctccacact gaagaggcgg cagcaggggg cgggcaactg cagcgtggcc
 541 agggccgagg cctgcatcaa gtgtctgggg acagcagacc acgggctggc ggccctacaga
 601 gcgtatagcc tgggtgctgg ggggttgggc tgcggcctgc cgctgctgct cagctggga
 661 gcctacggcg cctcggggc ggccgtgcta cgcagcccag gcatgactgt ggccgagaag
 721 ctgcgtgtgg cagcgttggt ggccagtggt ttggccctct acgcccagtc ctatgtgccc
 781 taccacatca tgcgggtgct caacgtggat gctcggcgcc gctggagcac ccgtgcccc
 841 agcttttcag acatagccca ggccacagca gccctggagc tggggcccta cgtgggctac
 901 caggtgatgc ggggcctcat tctgtgttcc acccttact ctacatggcc
 961 gcagtgcaca gcctgggctg ctgctgcccga cactgccccg gctacaggga cagctggaac
 1021 ccagaggacg ccaagagcac tggccaagcc ctgcccctca atgccacagc cgcccctaaa
 1081 ccgtcagagc cccagtcccc tgagctgagc caatga

26. The following sequence for the P2Y₁₁ receptor is derived from human placenta
(accession number AF030335) protein sequence.

MDRGAKSCPANFLAAADDKLSGFQGDFLWPILVVEFLVAVASNG
LALYRFSIPKQRPWHPAVVFSVQLAVSDLLCALTLPPLAAYLYPPKHWRYGEEACRLE
5 RFLFTCNLLGSVIFITCISLNRYLGIVHPFFARSHLRPKHAWAVSAAGWVLAALLAMP
TLSFSHLKRPPQQGAGNCSVARPEACIKCLGTADHGLAAYRAYSLVLAGLGCGLPLLLT
LAAYGALGRAVLRSPGMTVAEKL RVAALVASGVALYASSYVPYHIMRVLNVDARRRWS
TRCPSFADIAQATAALELGPYVG YQVMRGLMPLAF CVHPLL YMAAVPSLGCCCRHCPG
10 YRDSWNPEDAKSTGQALPLNATAAPKPSEPQSRELSQ

VII. CLAIMS

What is claimed is:

1. A method of modulating odor sensitivity in a subject, comprising administering a composition to the subject, wherein the composition is an agonist of a P2X or P2Y purinergic receptor.
2. The method of claim 1, wherein the P2X purinergic receptor is a P2X₁, P2X₃, P2X₄, P2X₅, or P2X₆ purinergic receptor.
3. The method of claim 1, wherein the P2Y purinergic receptor is a P2Y₁, P2Y₂, P2Y₄, P2Y₆, or P2Y₁₁ purinergic receptor.
4. The method of claim 1, wherein the concentration of the composition is less than or equal to 30 μ M, 20 μ M, 10 μ M, 5 μ M, 4 μ M, 3 μ M, 2 μ M, 1.6 μ M, 1 μ M, 0.5 μ M, 0.1 μ M, 0.05 μ M, or 0.01 μ M.
5. The method of claim 1, wherein the agonist is a P2X selective agonist.
6. The method of claim 5, wherein the P2X selective agonist is α , β -meATP, β , γ -meATP, BzATP
7. The method of claim 1 or 3, wherein the agonist is a P2Y selective agonist.
8. The method of claim 7, wherein the P2Y selective agonist is ADP, UTP, UTP γ S, UDP, 2Cl-ADP, 2MeSADP, ADP β S, ADP β F.
9. The method of claim 1, wherein the agonist is a non-selective agonist.
10. The method of claim 9, wherein the non-selective agonist is ATP, ATP γ S, 2MeSATP, Ap₄A.
11. The method of claim 1, wherein the agonist enhances the Ca²⁺ released from coapplication of an odor stimulant and the agonist
12. The composition of claim 11, wherein the agonist is β γ -methylene ATP.
13. The method of claim 1, wherein the agonist suppresses the Ca²⁺ released from the coapplication of an odor stimulant and the agonist.
14. The method of claim 13, wherein the agonist is UTP and ADP- β S.
15. The method of claim 1, wherein the agonist increases the ratio of observed co-application-evoked calcium transient over the sum of individual odor and P2 agonists peak amplitudes in a cell activation assay.
16. The method of claim 1, wherein the agonist decreases the ratio of observed co-application evoked calcium transient over the sum of individual odor and P2 agonists peak amplitudes in a cell activation assay.
17. The method of claim 15 or 16, wherein the ratio is 0.69 to .83.
18. The method of claim 17, wherein the agonist is ATP.

19. The method of claims 15 or 16, wherein the ratio is 0.72 to .92.
20. The method of claim 19, wherein the agonist is $\beta\gamma$ -methylene ATP.
21. The method of claims 15 or 16, wherein the ratio is 0.52 to .64.
22. The method of claim 21, wherein the agonist is ADP- β S.
23. A method of modulating odor sensitivity in a subject, comprising administering a composition to the subject, wherein the composition is an agonist of a P2X purinergic receptor.
24. A method of modulating odor sensitivity in a subject, comprising administering a composition to the subject, wherein the composition is an agonist of a P2Y purinergic receptor.
25. A method of screening for an agonist or antagonist of a purinergic receptor of the olfactory system, comprising
 - (a) contacting a purinergic receptor with a test compound;
 - (b) detecting intracellular calcium levels; and
 - (c) screening for a change in calcium levels as compared to a control level, wherein a change in the calcium level relative to a control indicates the compound is an agonist or an antagonist of a purinergic receptor of the olfactory system.
26. The method of claim 25, wherein the compound is an agonist.
27. The method of claim 25, wherein the compound is an antagonist.
28. The method of claim 25, wherein the change in calcium levels is transient.
29. The method of claim 25, wherein the change in calcium levels is sustained.
30. The method of claims 25, wherein the compound is selected when the calcium level increases as compared to a control level.
31. The method of claim 25, wherein compound is selected when the calcium level decreases as compared to a control level.
32. The method of claim 25, wherein the purinergic receptor is a P2X or a P2Y purinergic receptor.
33. The method of claim 25, wherein the P2X purinergic receptor is a P2X₁, P2X₃, P2X₄, P2X₅, or P2X₆ purinergic receptor.
34. The method of claim 25, wherein the P2Y purinergic receptor is a P2Y₁, P2Y₂, P2Y₄, P2Y₆, or P2Y₁₁ purinergic receptor.
35. The method of claim 25, further comprising the step of:
 - (d) screening for reversibility of response by removing the agonist or antagonist during the assay.
36. The method of claim 35, further comprising the step of:
 - (e) screening for dependence upon extracellular Ca²⁺ by repeating the assay in a solution devoid of extracellular Ca²⁺.
37. The method of claim 25, wherein the purinergic receptor is on olfactory epithelium.
38. The method of claim 35, wherein the olfactory epithelium comprises olfactory receptor neurons.
39. The method of claim 35, wherein the olfactory epithelium comprises sustentacular cells.

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40. The method of claim 25, wherein the calcium levels are detected using a calcium indicator.
41. The method of claim 35, wherein the calcium indicator is Fluo-4 AM.
42. The method of claim 35, wherein the calcium indicator is Fura-2/AM.
43. The method of claim 35, wherein the calcium indicator is Indo-1.
44. The method of claim 35, wherein the calcium indicator is or Indo-4.
45. A method of screening for an agonist of a purinergic receptor of the olfactory system, comprising
 - (a) contacting a purinergic receptor with a test compound;
 - (b) detecting intracellular calcium levels; and
 - (c) screening for a change in calcium levels as compared to a control level, wherein a change in the calcium level relative to a control indicates the compound is an agonist of a purinergic receptor of the olfactory system.
46. A method of screening for an antagonist of a purinergic receptor of the olfactory system, comprising
 - (a) contacting a purinergic receptor with a test compound;
 - (b) detecting intracellular calcium levels; and
 - (c) screening for a change in calcium levels as compared to a control level, wherein a change in the calcium level relative to a control indicates the compound is an antagonist of a purinergic receptor of the olfactory system.
47. A method of screening for an agonist or an antagonist of a purinergic receptor of the olfactory system, comprising
 - (a) contacting a first purinergic receptor cell with a set of test compounds ;
 - (b) detecting calcium levels in the first purinergic receptor cell; and
 - (c) selecting each compound in the set that contacted the first purinergic receptor cell, wherein the first purinergic receptor cell showed a transient change in calcium as compared to a control level, indicating the compound is an agonist or an antagonist of a purinergic receptor of the olfactory system.
48. The method of claim 35, further comprising the step:
 - (d) contacting a second purinergic receptor cell with one test compound selected in step (c).
49. The method of claim 35, further comprising the step:
 - (e) detecting calcium levels in the second purinergic receptor cell, wherein a transient change in calcium as compared to a control level indicates the compound is an agonist or an antagonist of a purinergic receptor of the olfactory system.
50. A method of screening for an agonist or an antagonist of a purinergic receptor of the olfactory system, comprising
 - (a) contacting a test compound with a cell that expresses a heterologous nucleic acid that encodes a purinergic receptor; and
 - (b) detecting calcium levels in the cell; a transient change in calcium as compared to a control level, indicating an agonist or an

antagonist of a purinergic receptor of the olfactory system..

51. A method of modulating odor sensitivity in a subject, comprising administering a composition to the subject, wherein the composition is an antagonist of a P2X or P2Y purinergic receptor.
52. The method of claim 51, wherein the P2X purinergic receptor is a P2X₁, P2X₃, P2X₄, P2X₅, or P2X₆ purinergic receptor.
53. The method of claim 51, wherein the P2Y purinergic receptor is a P2Y₁, P2Y₂, P2Y₄, P2Y₆, or P2Y₁₁ purinergic receptor.
54. The method of claim 51, wherein the odor sensitivity of the subject is increased.
55. The method of claim 51, wherein the antagonist is a P2X selective antagonist.
56. The method of claim 55, wherein the P2X selective antagonist is NF023, NF279 or KN-62a.
57. The method of claim 51, wherein the antagonist is a P2Y selective antagonist.
58. The method of claim 55, wherein the antagonist is ARL 67085, FPL 66096, A3P5PS, MRS 2179, 2-hexylthio-ATP, or 2-cyclohexylthio-ATP.
59. The method of claim 51, wherein the antagonist is a non-selective antagonist.
60. The method of claim 55, wherein the non-selective antagonist is Suramin, PPADS, Iso-PPADS, PSP, Reactive blue 2, Reactive Red, Trypan Blue, Evans Blue, or DIDS.
61. The method of claim 51, wherein the antagonist suppresses the Ca²⁺ released from coapplication of an odor stimulant and the antagonist.
62. The method of claim 51, wherein the agonist increases the ratio of observed co-application-evoked calcium transient over the sum of individual odor and P2 antagonists peak amplitudes in a cell activation assay.
63. A method of modulating odor sensitivity in a subject, comprising administering a composition to the subject, wherein the composition is an antagonist of a P2X purinergic receptor.
64. A method of modulating odor sensitivity in a subject, comprising administering a composition to the subject, wherein the composition is an antagonist of a P2Y purinergic receptor.
65. A method of protecting a cell from the effects of odor stimulation comprising administering a composition to the cell, wherein the composition is an antagonist or an agonist of a P2Y or a P2X purinergic receptor.
66. A method of modulating odor sensitivity in a subject, comprising inhibiting the interaction of ATP or ATP analog with a P2X or a P2Y purinergic receptor.

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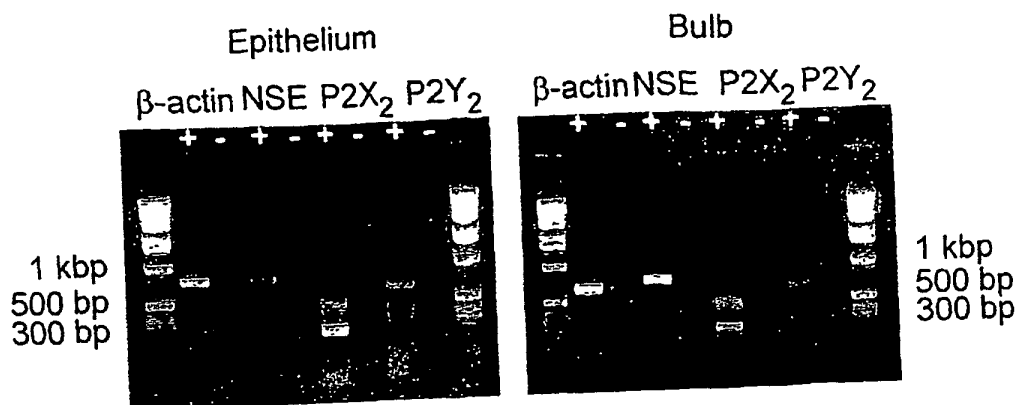


FIG.1A

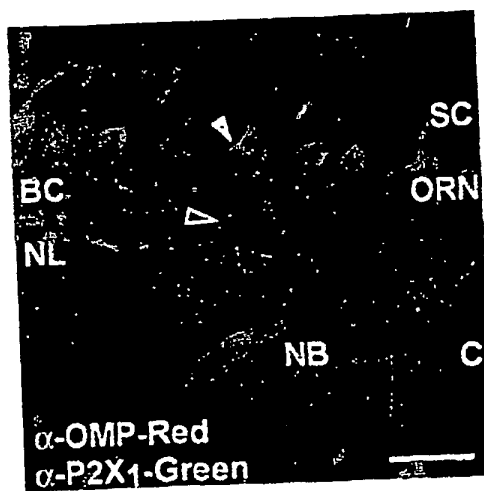


FIG.1B

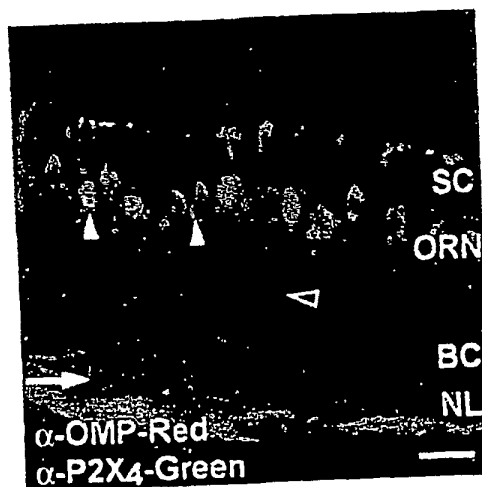
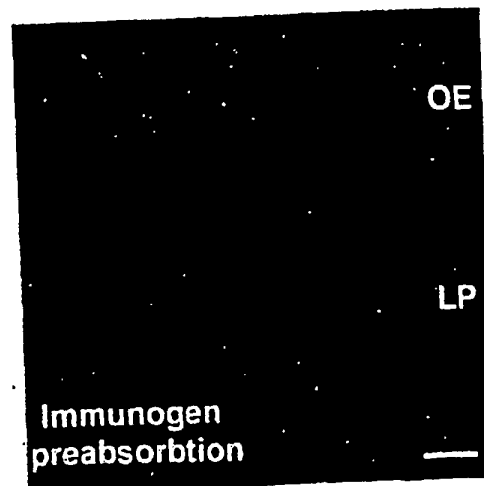
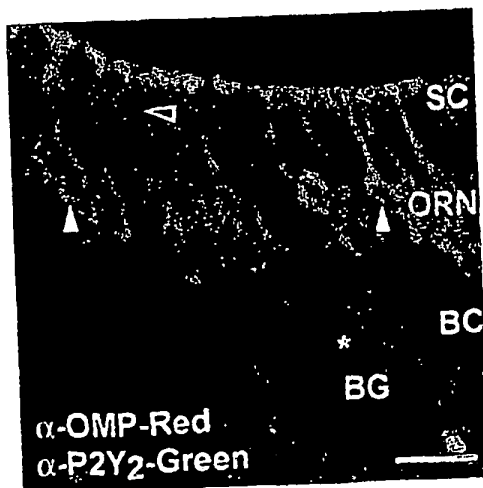


FIG.1C



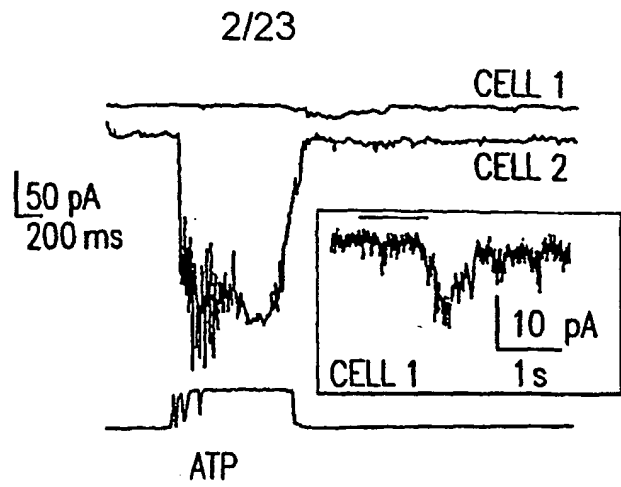


FIG.2A

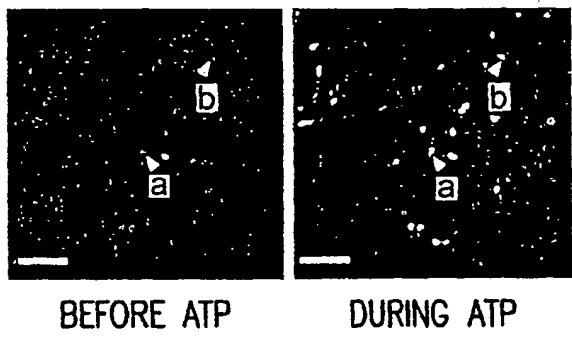


FIG.2B

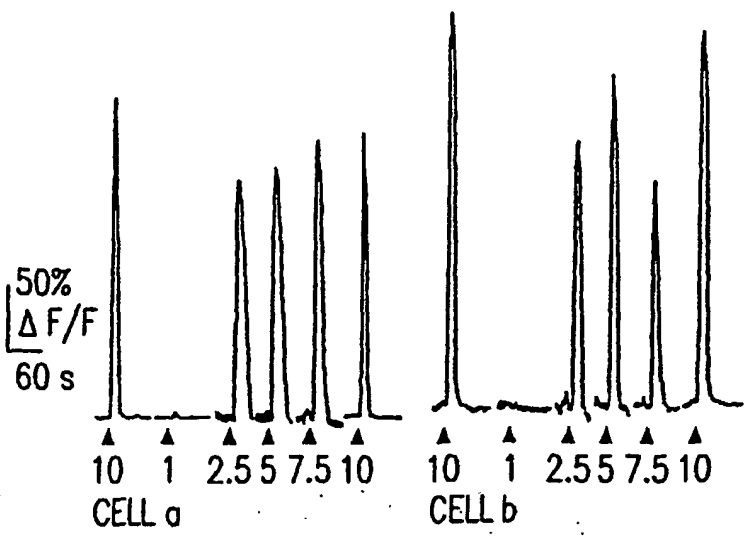


FIG.2C

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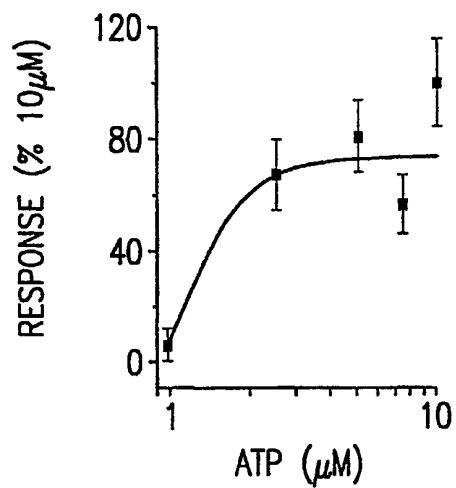


FIG.2D

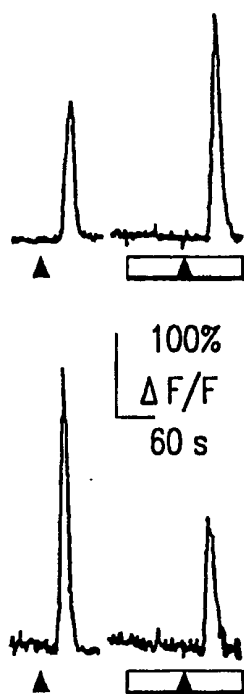


FIG.2E

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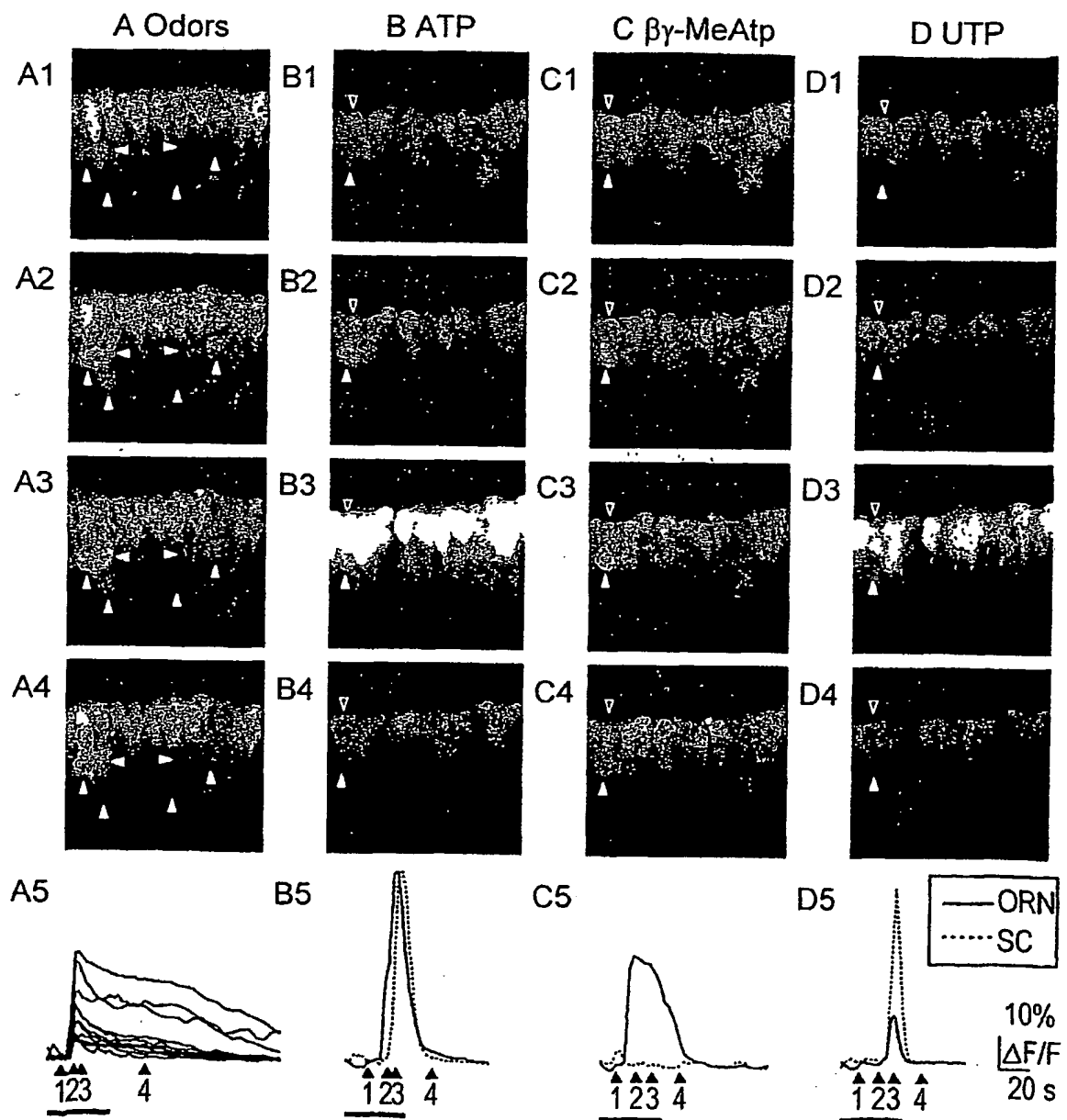


FIG.3

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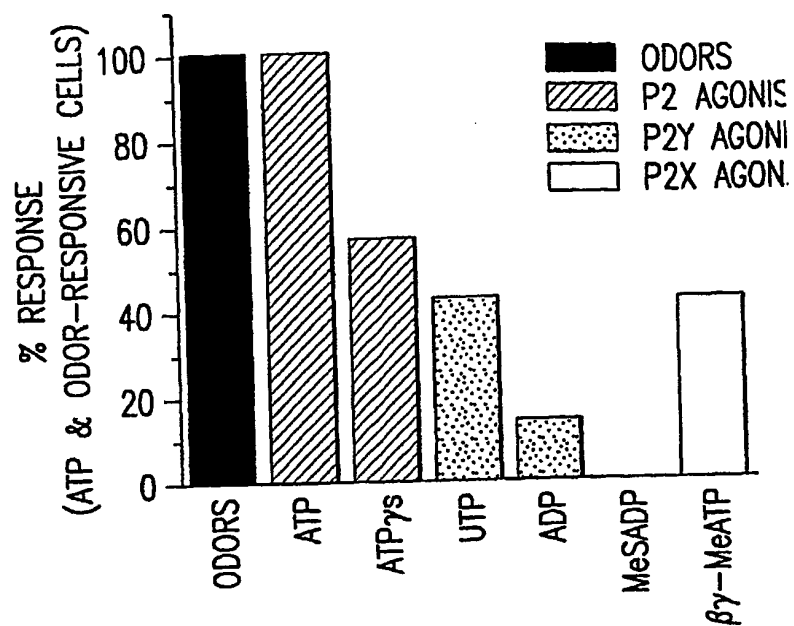


FIG. 4A

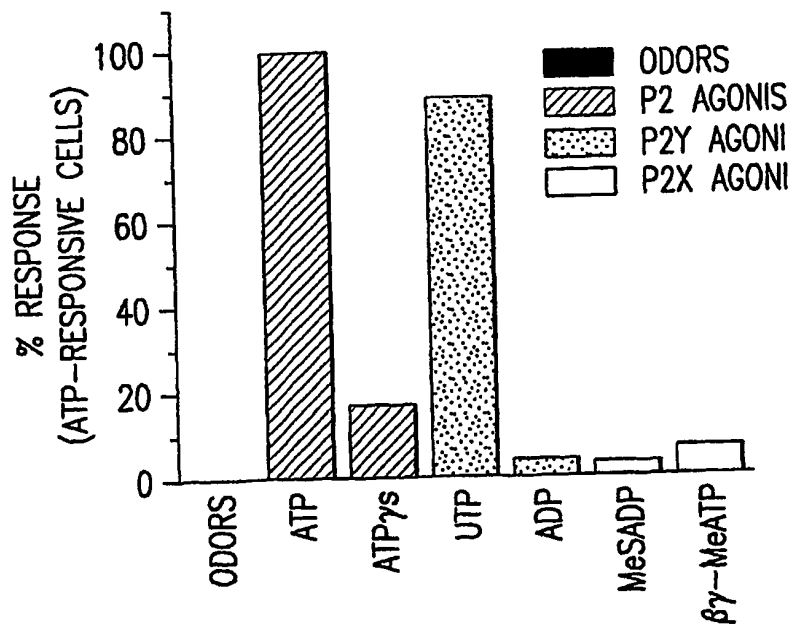


FIG. 4B

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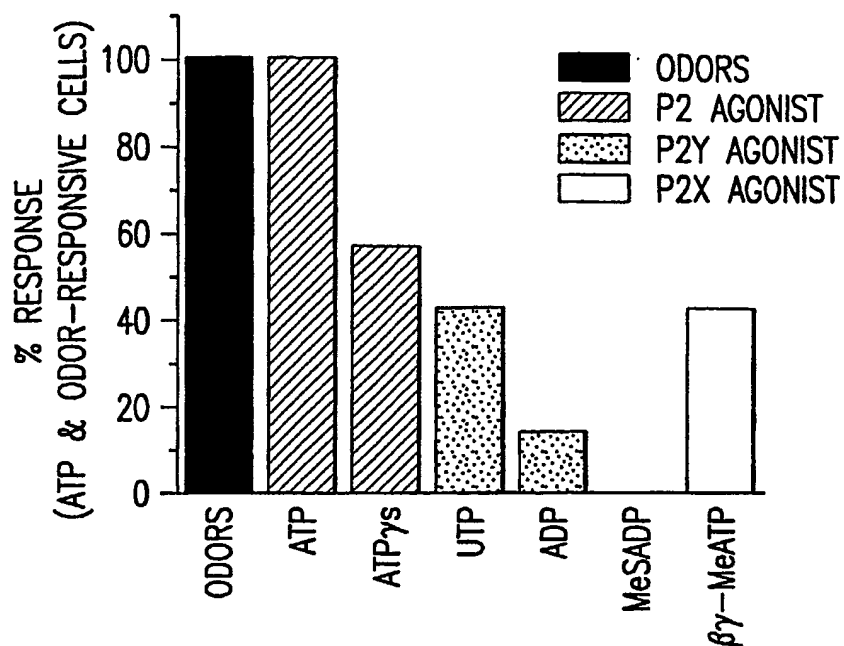


FIG. 4A

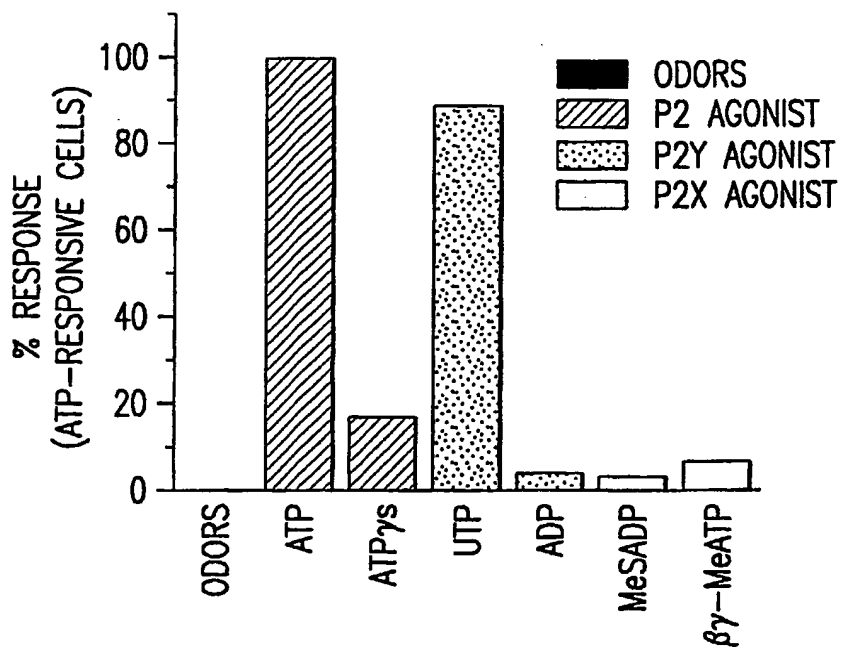


FIG. 4B

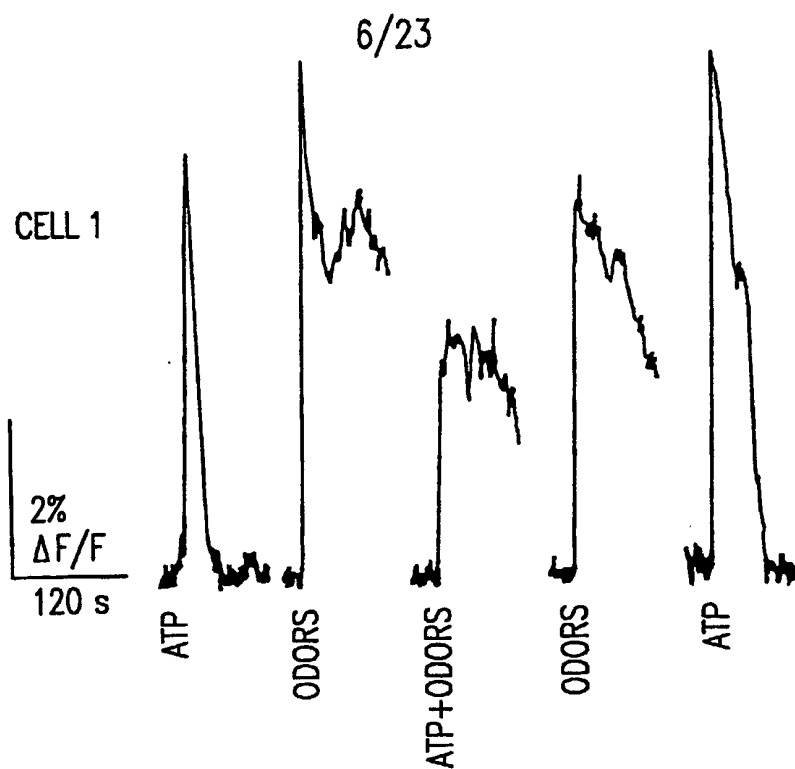


FIG. 5A

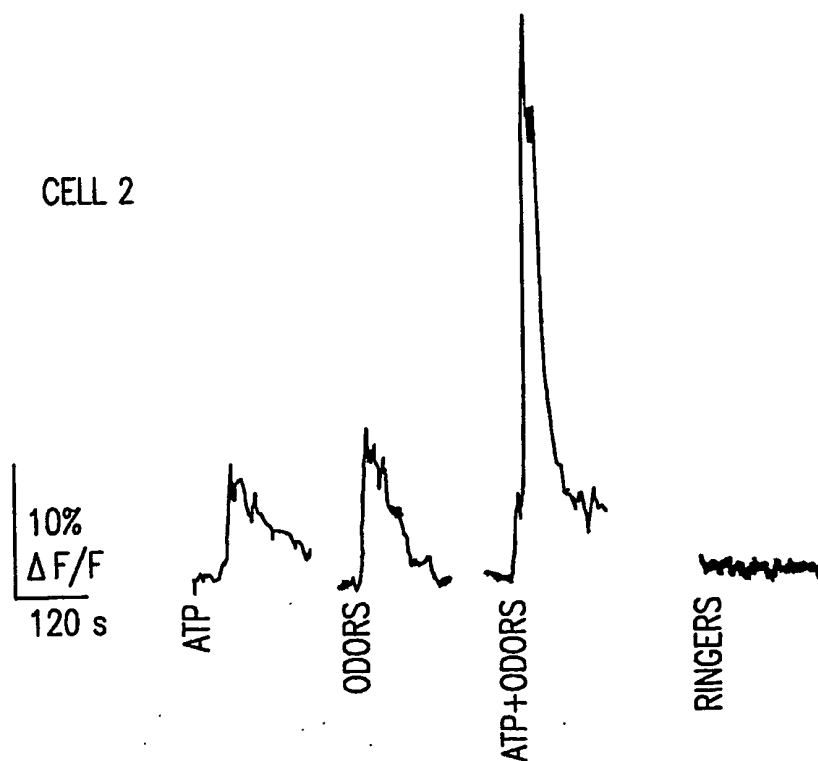


FIG. 5B

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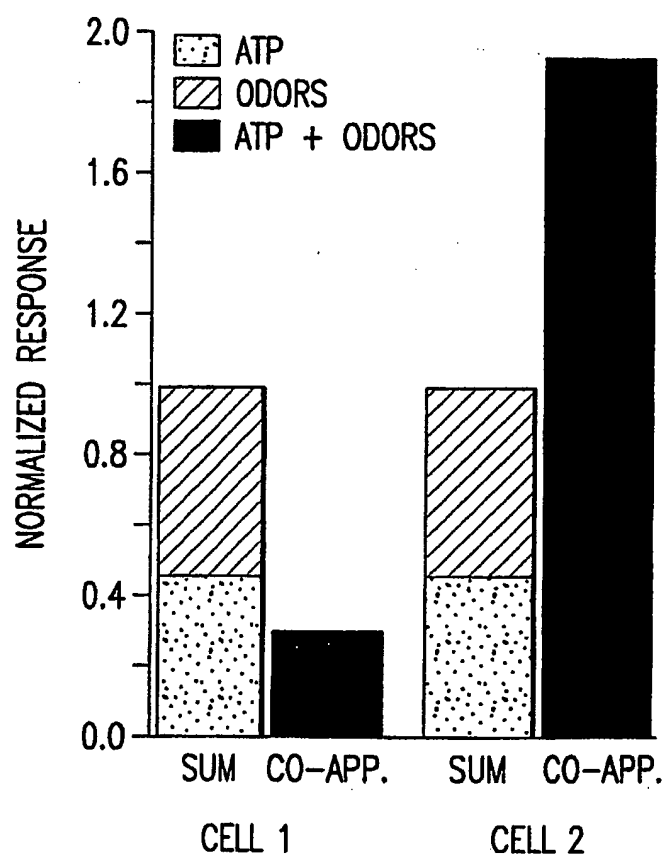


FIG.5C

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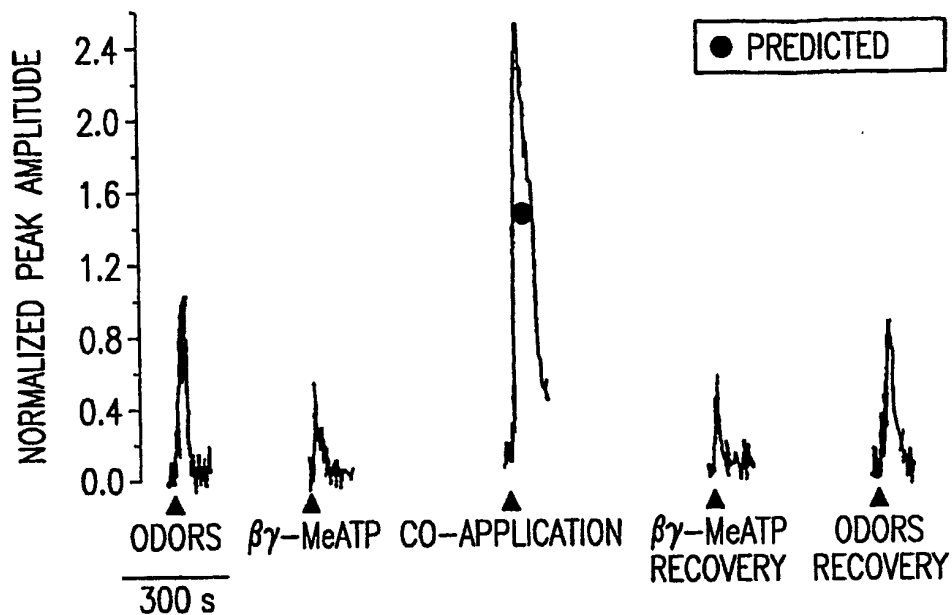


FIG. 6A

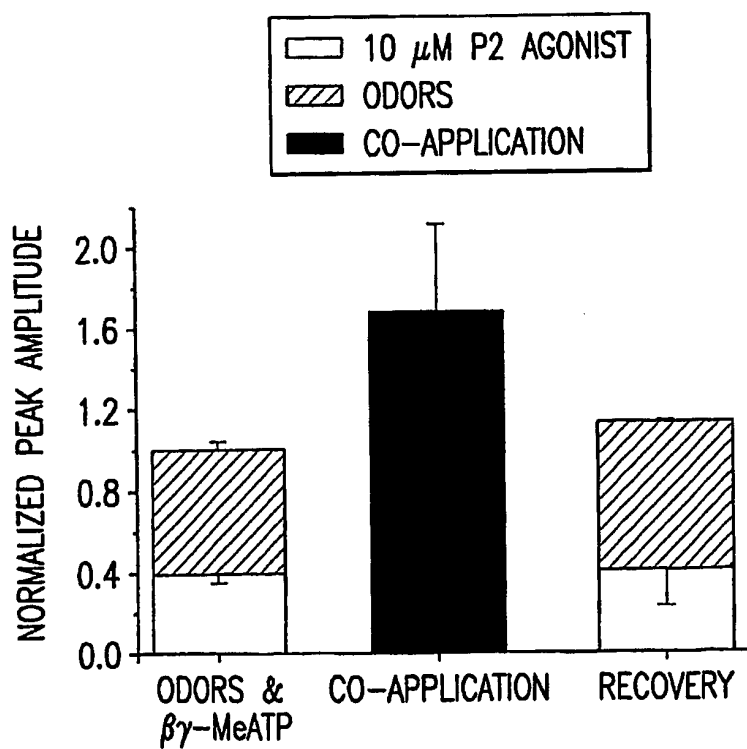


FIG. 6B

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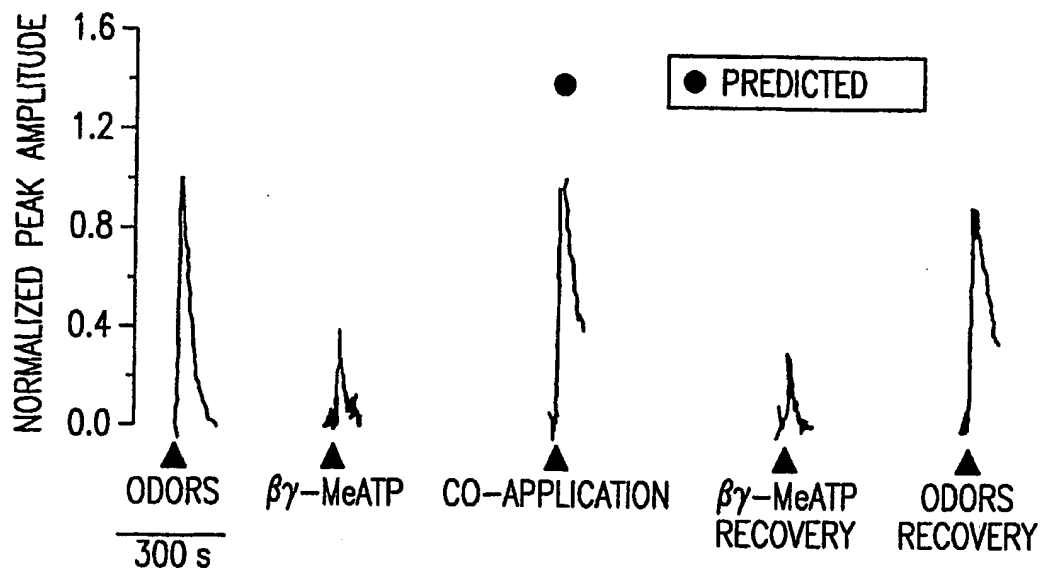


FIG. 6C

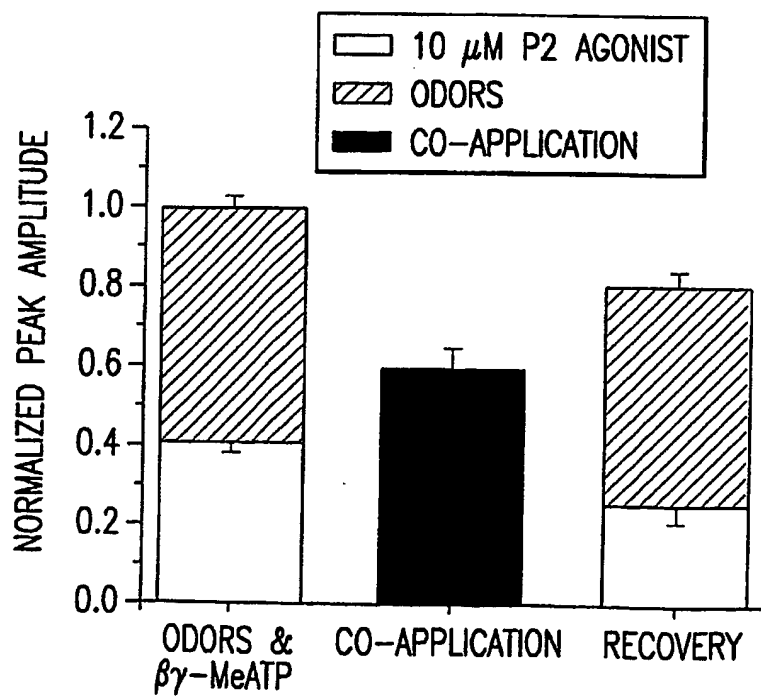


FIG. 6D

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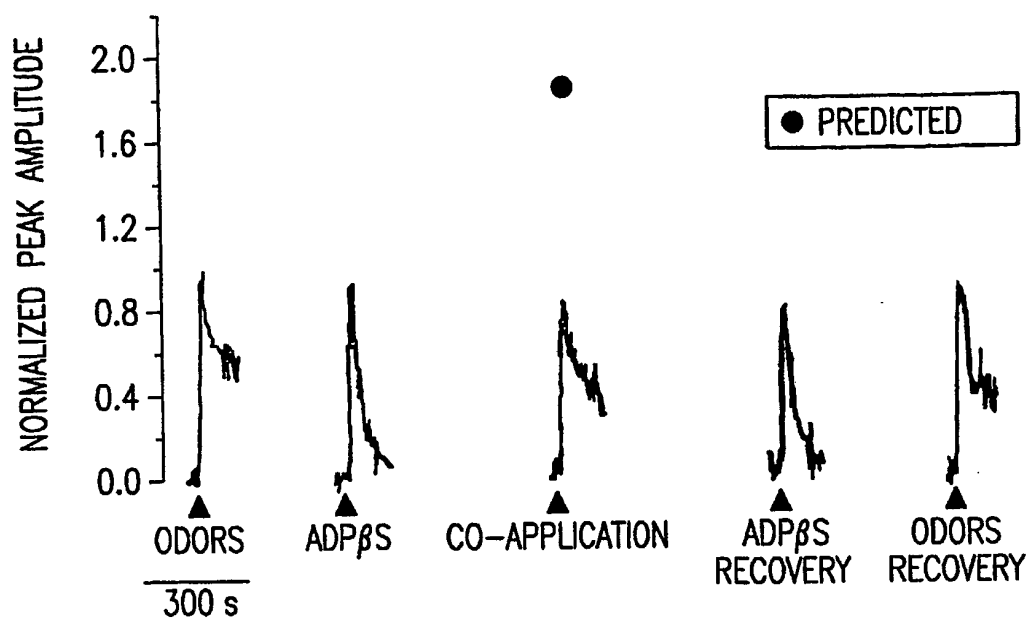


FIG.6E

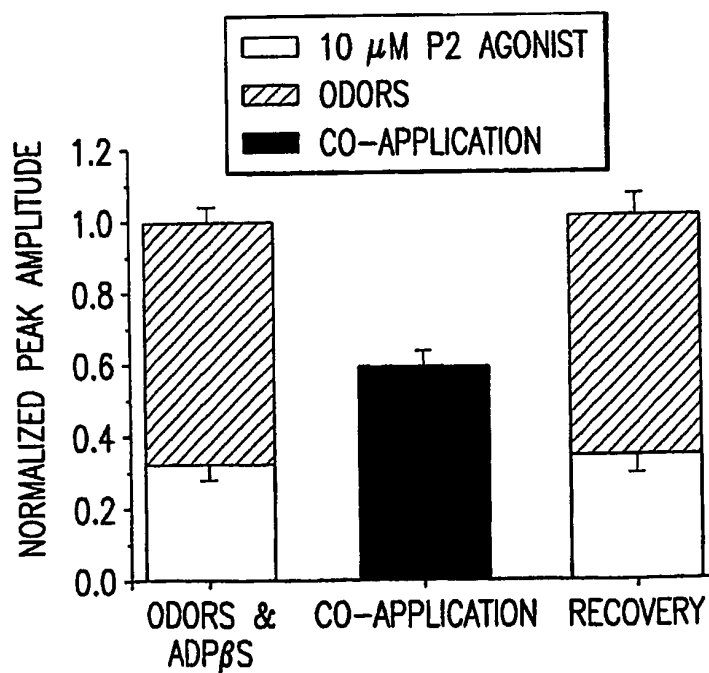


FIG.6F

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FIG. 7A

M1

P2X1	-MARRLQDELSAFFFEVDTPRMMLVRNKKVAVIFRLIQWLVMVIGWVFWKGGTSS	59
P2X2	-MRRRLARGCHSAFMDVETPKVIVRRRLQFVHRMQLILLVFWYVIVQSYQDSE	59
P2X3	-----MNCISDFFIVEITKSVVKSWTIGIINRAVQLIISYFVGNFLEKAYQVRD	53
P2X4	--MACCCSVLGSFLFEYDTPRIVLIRSRKVLINRAVQLILANVIGWVFWKGGTQD	58
P2X5	-MGQAANKGFVLSLFDYKTAKFVWAKSKKGLLYVQLIILLVLLIWFELIKSYQDID	59
P2X6	MASAVAAALVSMGFLDYKTEKYMTNRCWVGISQRLQLGWVMVIGWALLAKKGGQEND	60
*		
P2X1	DLISSVSV-RKGLAVTQLQGLPQVMDVADVMPAHGDSFVMTNFIVTPQQTQGHCA	118
P2X2	TGPESIIITAKGITMSDKV-----MDVEEYMKPPEGGSWSIIRIEVTPSQTLGTCP	114
P2X3	TAIESSVTKMGFG-----RYANRVMDSDYMPQGTSVFVIITKIIMTENCQMGFCP	108
P2X4	SWSSVTT-KAKGVAVNTSQLGRIMDVADVMPAQEENSIFIMTMMIVTNAQTQSTCP	117
P2X5	TSLQSAVTKMGVAYTNTMLGERLMDVADVMPDSQGENVFFWITNLIVTPNCRGGICA	119
P2X6	MDPQISVITKIKGVSVTQVKELEKRLMDVADVMPDSQGENVFFLVITNFMVTPAQVQGRCP	120
*		
P2X1	ENPE--GGIQDDSGTIPGKAERKAGIRTCNVPFNGTVK-ICEIFGMCVMEVDDKIPS	175
P2X2	ESMRVHSSSTCHSDDDIAGQLDMGNGIRTCVPTYHGDSKITCEVSAWCPVEDG-TSDN	173
P2X3	ENEE--KYRCVSDSQGPER--FPGGILITGRCNYSVLR-ICEIQGWCPTEND-TVEM	162
P2X4	ETPD-KTSCNLSADDTPLGRDTHSSGVAIGRCVFPNESVK-ICEVAWCPVENDVGPT	175
P2X5	EREGIPDGECSDDCHAGESVWAGHLKITGRQVRGNSTRGTCEIFAWCPVETK-SMPT	178
P2X6	EHPVPLANDWADEDPEGEMGTYSHGKITGCGQVAFNGTHR-ICEIWSMCVPESS-AVPR	178
*		
P2X1	PALLREAEVETLFIKNSISFPFQVNRRLVEEVNGTYMKQLYHKIQHPLCFVFNLGW	235
P2X2	HFLGKMAPNFIIILIKNSITHYPKFESKGNIASQKSDYLKH-QTFDDSDPYCFIFRLGFI	232
P2X3	P-IMMEAEVETLFIKNSIRFPLFNFEGNLLPNLTDKDIKCRFHPEKAPFCFILLRVGV	221
P2X4	PAFLKAENETLIVKNNIWPKNFSKRNLIPNITTSYLKSCQVNAQIDPFCHIFRLGTI	235
P2X5	DPLLKDAESFTIISIKNFIRFPKFNFSKANVLETDNKHFLLKTHF-SSTNLVCFIFRLGSI	237
P2X6	KPLLAKAKNETLFIKNTVTFNKENFSRTVALDTHDNTYFKQLYDSLSSPYCFVFRIGDL	238

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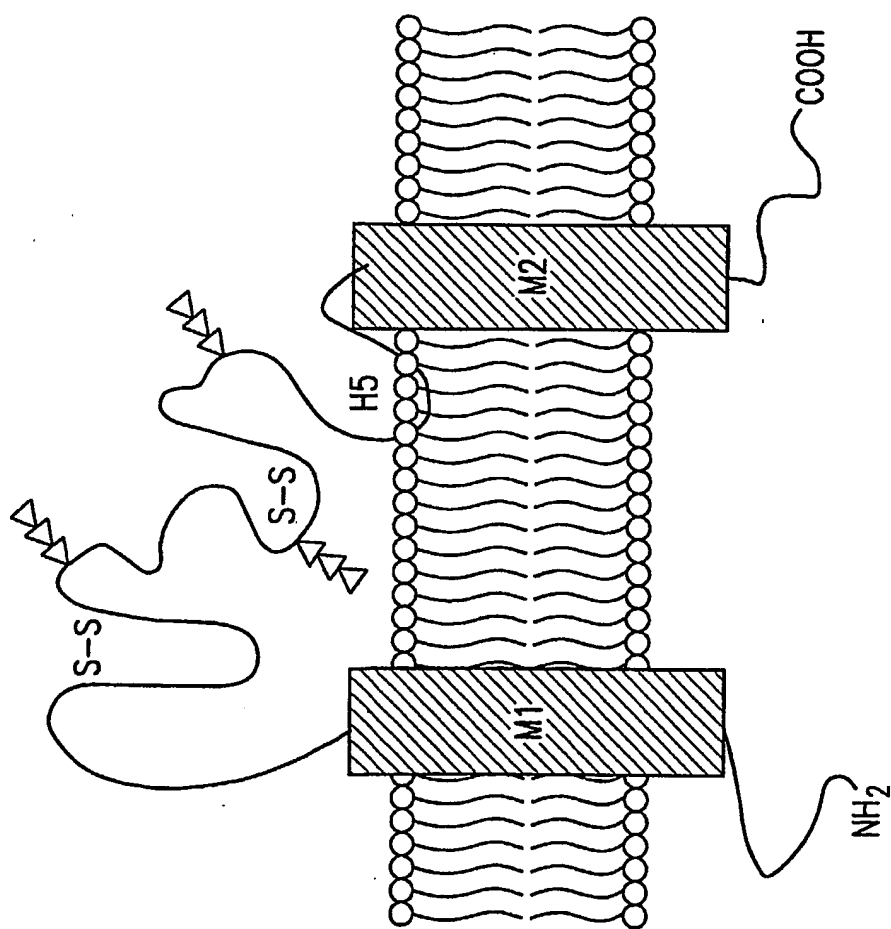


FIG.8

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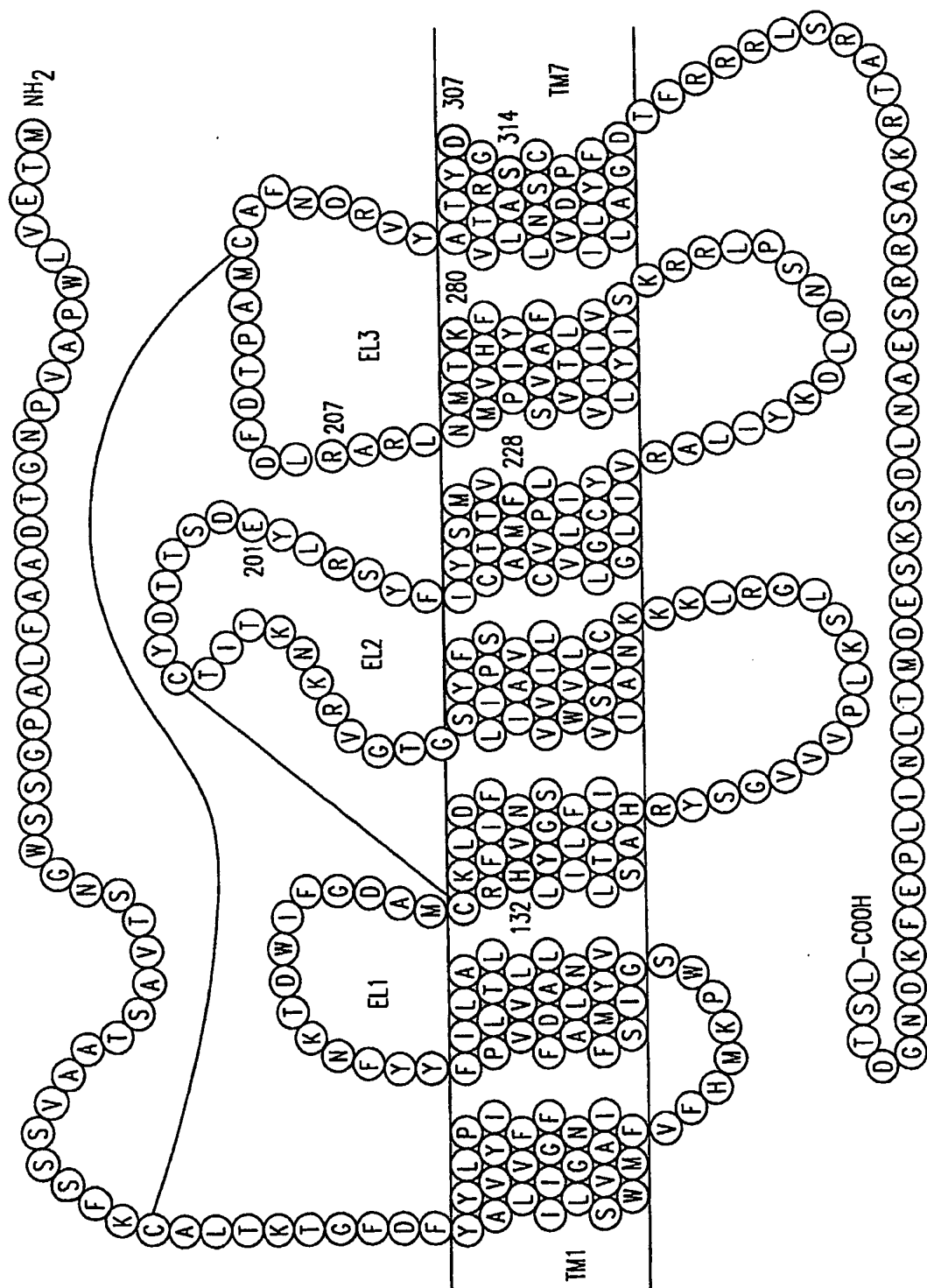


FIG. 9

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TM 3

P2Y1 (ADP)	125	KLQRFIFHVNLYGSILFLTCISAHR	149
P2Y2 (UTP)	107	KLVRFLFYTNLYCSILFLTCISVHR	131
P2Y4 (UTP)	109	KFVRFLFYWNLYCSVLFLTCISVHR	133
P2Y6 (UDP)	100	RLVRFLFYANLAGSILFLTCISFQR	124
P2Y11 (ATP)	100	RLERFLFTCNLLCSVIFITCISLNR	124

TM 5

P2Y1 (ADP)	215	FIYSMCTIVAMFCVFLVLILGCYGLIV	241
P2Y2 (UTP)	196	VAYSSVNLGLLFAVFFAVILVCYVLM	222
P2Y4 (UTP)	198	VKFSSAVMGLLFGVFCLVTLVCYGLMA	224
P2Y6 (UDP)	190	MPYGMALTVICFLLFFAALLACYCLLA	215
P2Y11 (ATP)	200	RAYSLVLAGLGCGLFLLTLAAYGALG	226

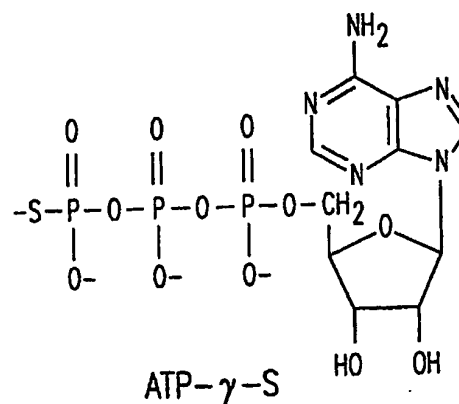
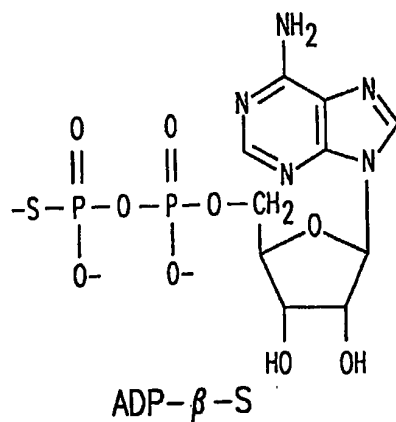
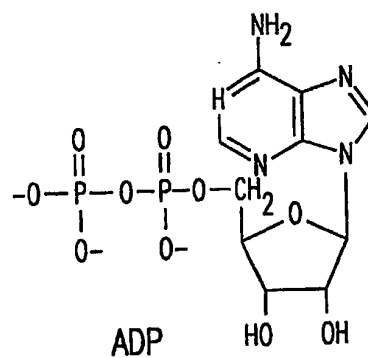
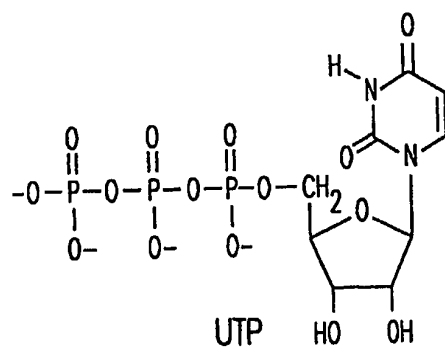
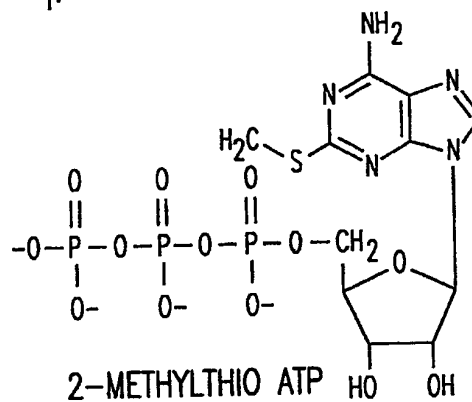
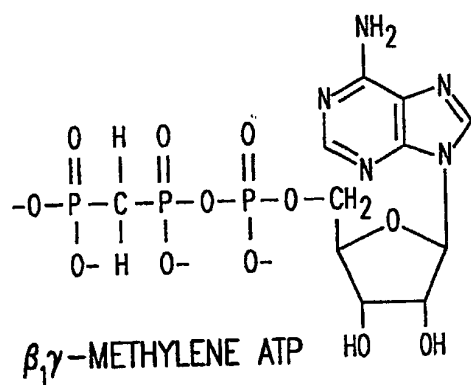
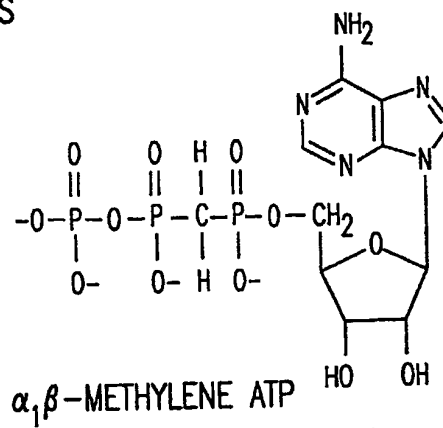
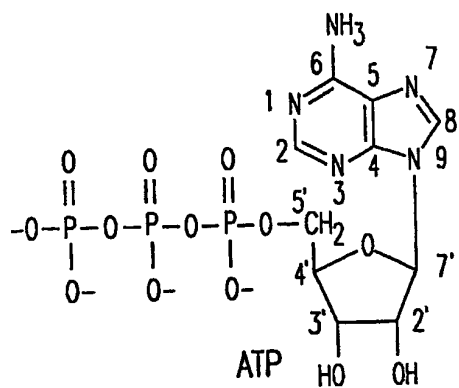
TM 6

P2Y1 (ADP)	260	YLVIIIVLTVFAVSYPHVMKTMNLR	285
P2Y2 (UTP)	245	RTIAVVLAVFALCFLPFHVTRTLYY	270
P2Y4 (UTP)	245	RTIAVVLTVFAVCFVPFHITRTIYYL	270
P2Y6 (UDP)	239	RMAVVVAAFAISFLPFHITKTAYLA	264
P2Y11 (ATP)	245	ALVASGVALYASSYVPYHIMRVINVD	270

TM7

P2Y1 (ADP)	303	YATYQVTRGLASLNSCVDPILYPLAGDT	330
P2Y2 (UTP)	285	NMAYKVTRFLASANSCLDPVLYFLAGQR	312
P2Y4 (UTP)	285	NVYKVTRFLASANSCLDPVLYLLTGDK	312
P2Y6 (UDP)	280	AAAYKGTRFFASANSVLDPIIFYFTQKK	307
P2Y11 (ATP)	297	YVGYQVMRGLMPLASCVHPLLYMAAUPS	324

FIG.10

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AGONISTS

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AGONISTS (CONT'D)

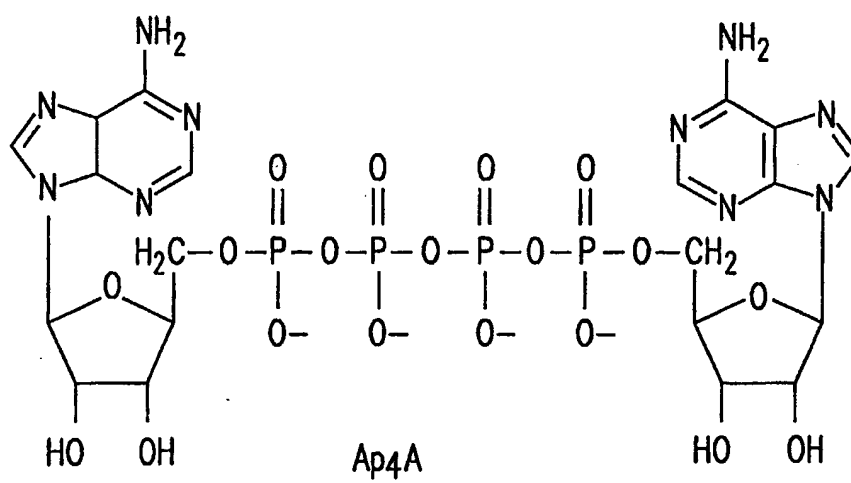


FIG.11B

ANTAGONISTS

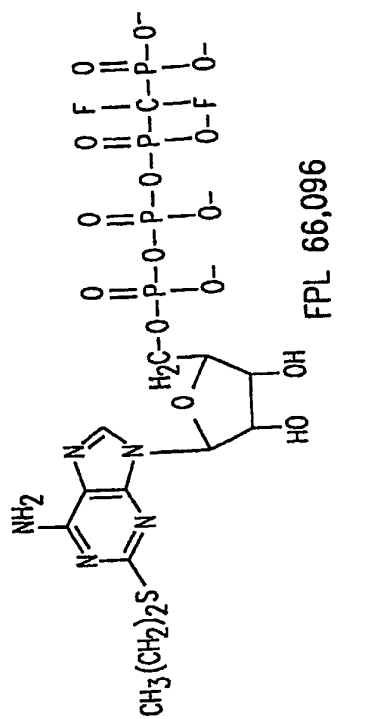
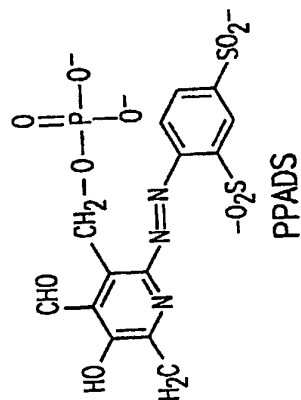
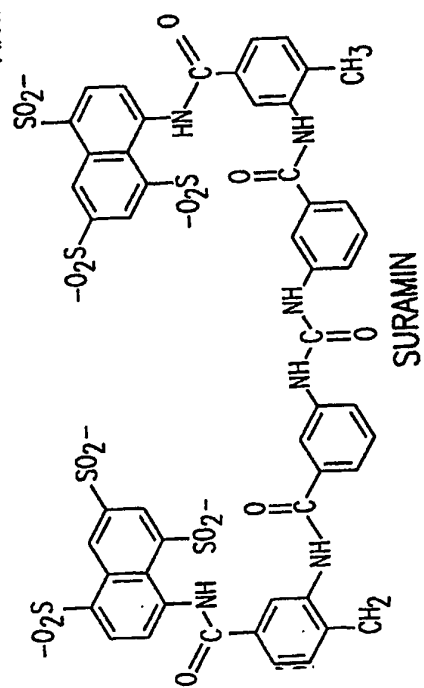


FIG. 11C

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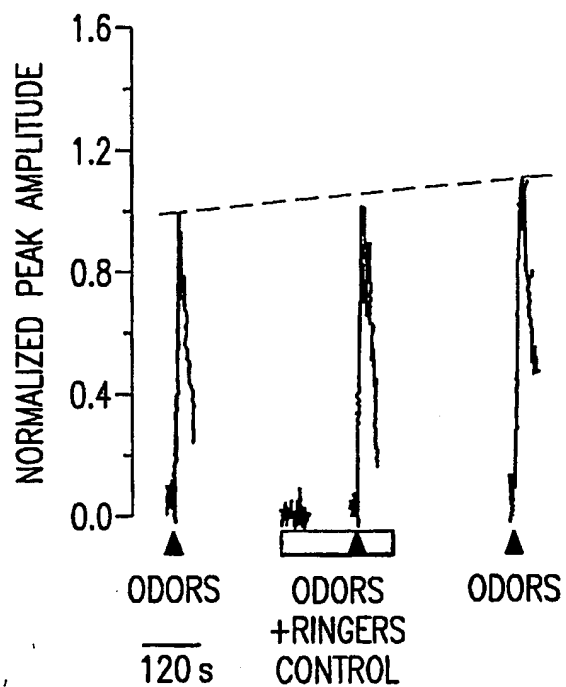


FIG.12A

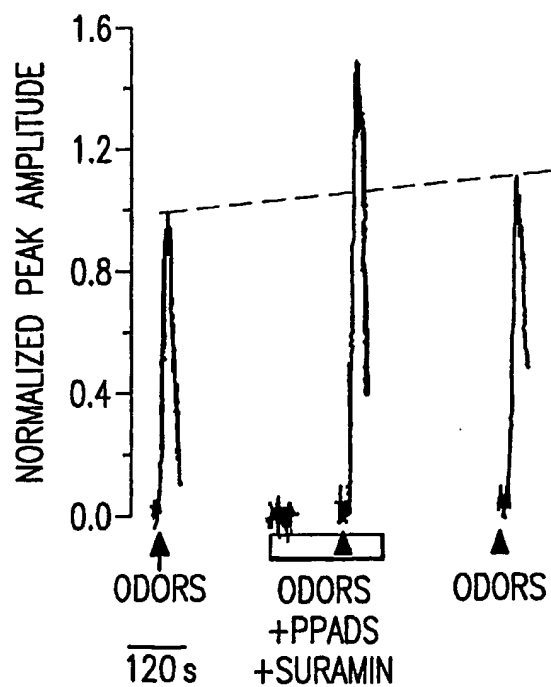


FIG.12B

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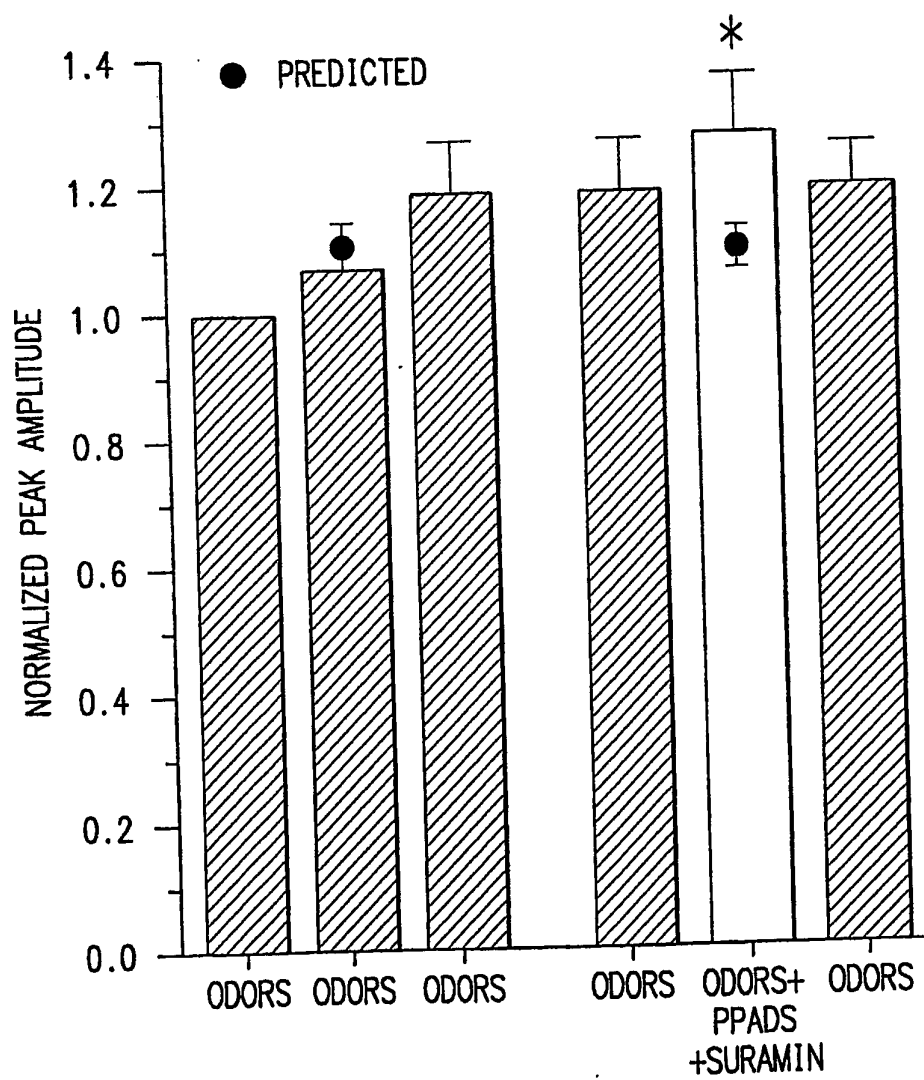


FIG.12C

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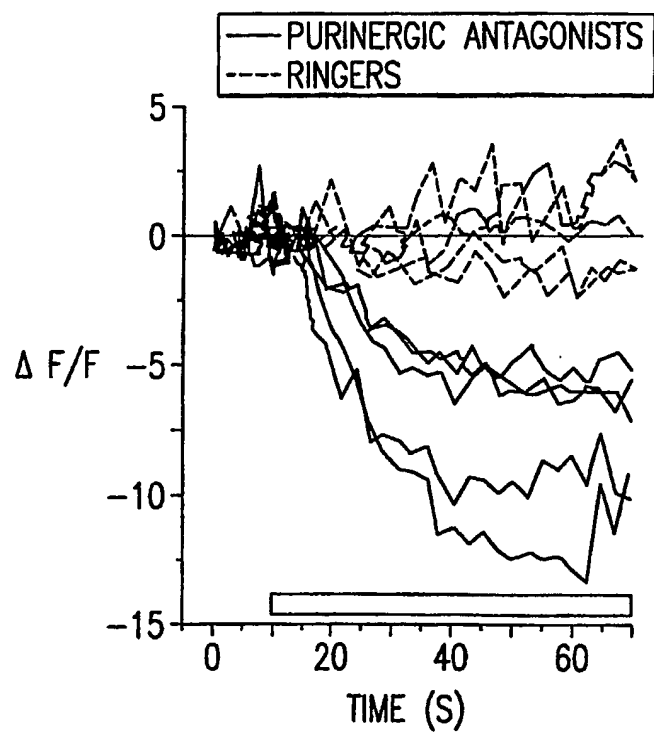


FIG.12D

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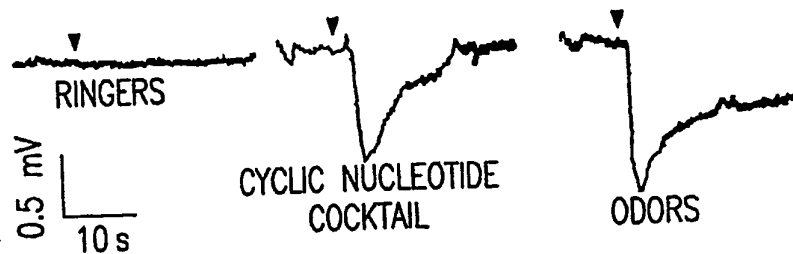


FIG.13A

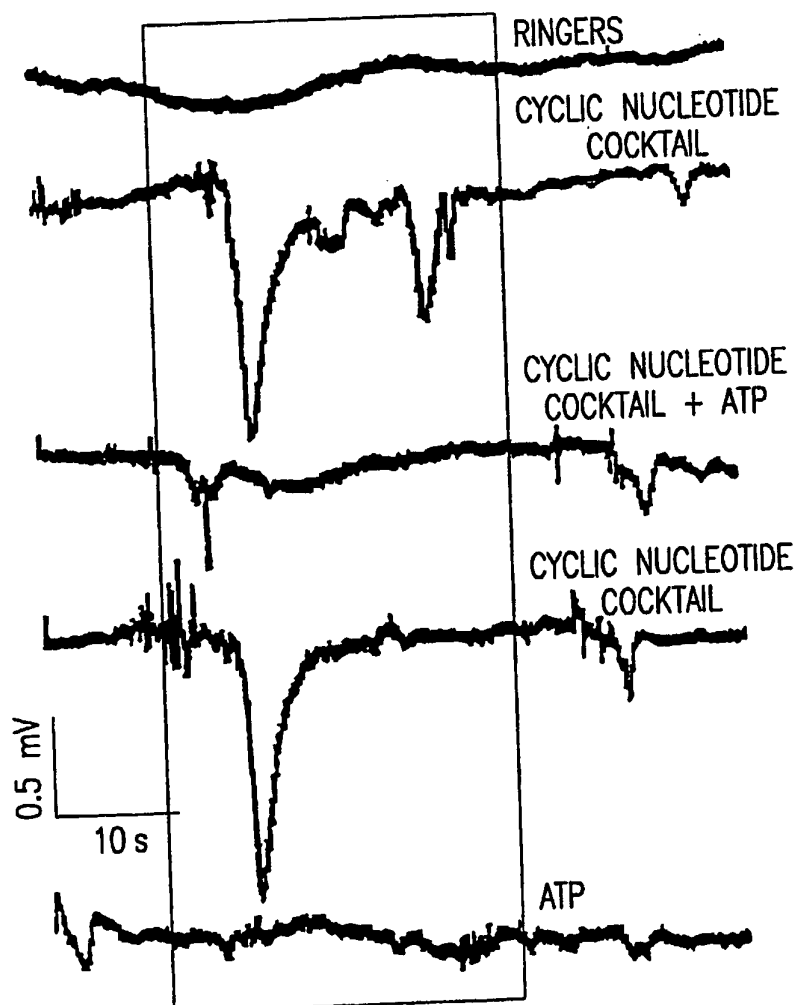


FIG 13B

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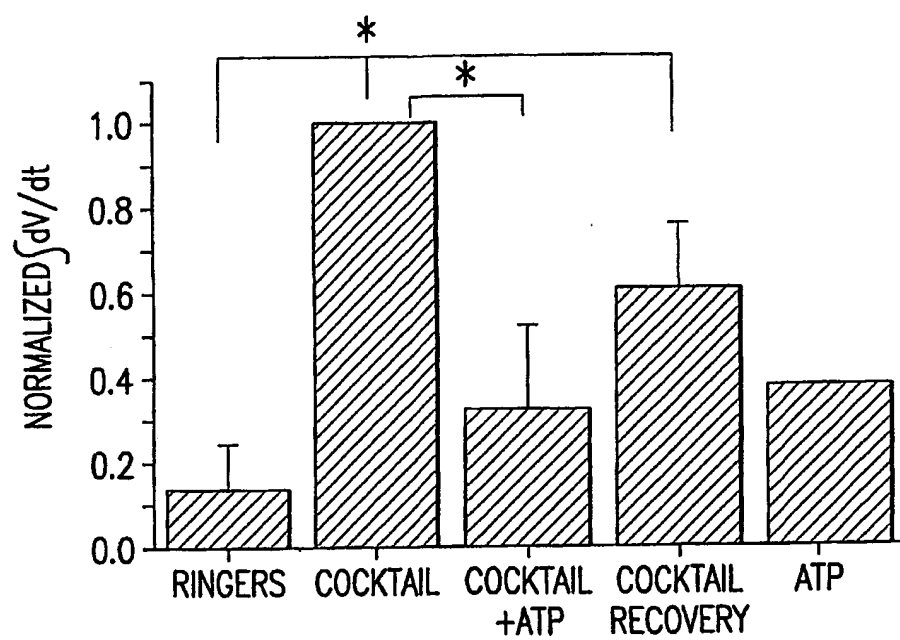


FIG.13C

SEQUENCE LISTING

<110> University of Utah Research Foundation

Lucero, Mary
Hegg, Colleen

<120> Purinergic Modulation of Smell

<130> 21101.0030P1

<150> 60/428,140

<151> 2002-11-21

<160> 26

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 1452

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 1

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ctacgacagg ctggggggctt ctggattata agacggagaa gtatgtgatg accaggaact 120
ggcgggtggg cgccctgcag aggctgctgc agtttgggat cgtgggtctat gtggtagggg 180
gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaaccccag ttttccatca 240
tcaccaaact caaagggggt tccgtcactc agatcaagga gcttggaaac cggctgtggg 300
atgtggccga cttcgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact 360
tccttgtgac gccagcccaa gttcagggca gatgccaga gcaccgtcc gtcccactgg 420
ctaactgctg ggtcgacgaa gactgcccag aaggggaggg aggcacacac agccacggtg 480
taaaaacagg ccagtgtgtg gtgttcaatg ggaccacag gacctgtgag atctggagt 540
ggtgcccagt ggagagtggc gttgtgccct cgaggcccct gctggcccag gccagaaact 600
tcacactgtt catcaaaaac acagtcacct tcagcaagtt caacttctct aagtccaatg 660
ccttggagac ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc 720
cctactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg 780
acctggcggt gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca 840
ccggggactc tggtgctggg cctcactact ccttcagct gcaggagaag agctacaact 900
tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgcacc ctgctcaagc 960
tctatggaat ccgcttcgac atcctcgtca ccgggcaggc agggaagttc gggctcatcc 1020
ccacggccgt cacttgggc accggggcag cttggctggg cgtggtcacc tttttctgtg 1080
acctgtact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg 1140
aggccaaggc ccgaaagca accgccaact ctgtgtggag ggagctggcc ttgcatccc 1200
aagcccgact ggccgagtgc ctcagacgga gctcagcacc tgcaccacg gccactgctg 1260
ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa 1320
ccattccgg gagcctgtag ccgtttccct gctggttgag aagagagagg ggctgggcaa 1380
ggaaggaccc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat 1440
caagccgaat tc                                     1452

```

<210> 2

<211> 399

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 2

```

Met Ala Arg Arg Phe Gln Glu Glu Leu Ala Ala Phe Leu Phe Glu Tyr
 1           5           10           15
Asp Thr Pro Arg Met Val Leu Val Arg Asn Lys Lys Val Gly Val Ile
      20           25           30
Phe Arg Leu Ile Gln Leu Val Val Leu Val Tyr Val Ile Gly Trp Val
      35           40           45
Phe Leu Tyr Glu Lys Gly Tyr Gln Thr Ser Ser Gly Leu Ile Ser Ser
      50           55           60
Val Ser Val Lys Leu Lys Gly Leu Ala Val Thr Gln Leu Pro Gly Leu
      65           70           75           80
Gly Pro Gln Val Trp Asp Val Ala Asp Tyr Val Phe Pro Ala Gln Gly
      85           90           95
Asp Asn Ser Phe Val Val Met Thr Asn Phe Ile Val Thr Pro Lys Gln
      100          105          110
Thr Gln Gly Tyr Cys Ala Glu His Pro Glu Gly Gly Ile Cys Lys Glu
      115          120          125
Asp Ser Gly Cys Thr Pro Gly Lys Ala Lys Arg Lys Ala Gln Gly Ile
      130          135          140
Arg Thr Gly Lys Cys Val Ala Phe Asn Asp Thr Val Lys Thr Cys Glu
      145          150          155          160
Ile Phe Gly Trp Cys Pro Val Glu Val Asp Asp Asp Ile Pro Arg Pro
      165          170          175
Ala Leu Leu Arg Glu Ala Glu Asn Phe Thr Leu Phe Ile Lys Asn Ser
      180          185          190
Ile Ser Phe Pro Arg Phe Lys Val Asn Arg Arg Asn Leu Val Glu Glu
      195          200          205
Val Asn Ala Ala His Met Lys Thr Cys Leu Phe His Lys Thr Leu His
      210          215          220
Pro Leu Cys Pro Val Phe Gln Leu Gly Tyr Val Val Gln Glu Ser Gly
      225          230          235          240
Gln Asn Phe Ser Thr Leu Ala Glu Lys Gly Gly Val Val Gly Ile Thr
      245          250          255
Ile Asp Trp His Cys Asp Leu Asp Trp His Val Arg His Cys Arg Pro
      260          265          270
Ile Tyr Glu Phe His Gly Leu Tyr Glu Glu Lys Asn Leu Ser Pro Gly
      275          280          285
Phe Asn Phe Arg Phe Ala Arg His Phe Val Glu Asn Gly Thr Asn Tyr
      290          295          300
Arg His Leu Phe Lys Val Phe Gly Ile Arg Phe Asp Ile Leu Val Asp
      305          310          315          320
Gly Lys Ala Gly Lys Phe Asp Ile Ile Pro Thr Met Thr Thr Ile Gly
      325          330          335
Ser Gly Ile Gly Ile Phe Gly Val Ala Thr Val Leu Cys Asp Leu Leu
      340          345          350
Leu Leu His Ile Leu Pro Lys Arg His Tyr Tyr Lys Gln Lys Lys Phe
      355          360          365
Lys Tyr Ala Glu Asp Met Gly Pro Gly Ala Ala Glu Arg Asp Leu Ala
      370          375          380
Ala Thr Ser Ser Thr Leu Gly Leu Gln Glu Asn Met Arg Thr Ser
      385          390          395

```

<210> 3

<211> 1452

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 3

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gaattcggct gatccccggg caggtgctag caggagctgg cagcatgggc tccccagggg 60
ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact 120
ggcgggtggg cgccctgcag aggtctgtgc agtttgggat cgtggtctat gtggtagggt 180
gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaaccccag ttttccatca 240
tcaccaaact caaagggggt tccgtcactc agatcaagga gcttggaac cggtgtggg 300
atgtggccga cttcgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact 360
tccttgtgac gccagcccaa gttcagggca gatgccca gaacccgtcc gtcccactgg 420
ctaaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacgggtg 480
taaaaacagg ccagtgtgtg gtgttcaatg ggaccacag gacctgtgag atctggagtt 540
gggtgccagt ggagagtggc gttgtgccct cgaggccct gctggcccag gccagaact 600
tcacactgtt catcaaaaac acagtcaact tcagcaagtt caacttctct aagtccaatg 660
ccttgagagc ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc 720
cctactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg 780
acctggcggt gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca 840
ccggggactc tggctgtgtg cctcactact ccttcagct gcaggagaag agctacaact 900
tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgacc ctgctcaagc 960
tctatggaat ccgcttcgac atcctcgtca ccgggcaggc agggaaagttc gggctcatcc 1020
ccacggccgt cacactgggc accggggcag cttggctggg cgtggtcacc ttttctgtg 1080
acctgctact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg 1140
aggccaaggg cccgaaagca accgccaact ctgtgtggag ggagctggcc tttgcatccc 1200
aagcccgact ggccgagtgc ctcagacgga gctcagacc tgcaccacg gccactgctg 1260
ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa 1320
cccattccgg gagcctgtag ccgtttccct gctggttgag aagagagagg ggctgggcaa 1380
ggaaggacc ctgccctgcc gagcgaaagc aagatgagg caacagcaat gaaagaagat 1440
caagccgaat tc                                     1452

```

<210> 4

<211> 472

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 4

```

Met Val Arg Arg Leu Ala Arg Gly Cys Trp Ser Ala Phe Trp Asp Tyr
1      5      10      15
Glu Thr Pro Lys Val Ile Val Val Arg Asn Arg Arg Leu Gly Phe Val
20     25     30
His Arg Met Val Gln Leu Leu Ile Leu Leu Tyr Phe Val Trp Tyr Val
35     40     45
Phe Ile Val Gln Lys Ser Tyr Gln Asp Ser Glu Thr Gly Pro Glu Ser
50     55     60
Ser Ile Ile Thr Lys Val Lys Gly Ile Thr Met Ser Glu Asp Lys Val
65     70     75     80
Trp Asp Val Glu Glu Tyr Val Lys Pro Pro Glu Gly Gly Ser Val Val
85     90     95
Ser Ile Ile Thr Arg Ile Glu Val Thr Pro Ser Gln Thr Leu Gly Thr
100    105    110
Cys Pro Glu Ser Met Arg Val His Ser Ser Thr Cys His Ser Asp Asp
115    120    125
Asp Cys Ile Ala Gly Gln Leu Asp Met Gln Gly Asn Gly Ile Arg Thr
130    135    140

```

Gly His Cys Val Pro Tyr Tyr His Gly Asp Ser Lys Thr Cys Glu Val
 145 150 155 160
 Ser Ala Trp Cys Pro Val Glu Asp Gly Thr Ser Asp Asn His Phe Leu
 165 170 175
 Gly Lys Met Ala Pro Asn Phe Thr Ile Leu Ile Lys Asn Ser Ile His
 180 185 190
 Tyr Pro Lys Phe Lys Phe Ser Lys Gly Asn Ile Ala Ser Gln Lys Ser
 195 200 205
 Asp Tyr Leu Lys His Cys Thr Phe Asp Gln Asp Ser Asp Pro Tyr Cys
 210 215 220
 Pro Ile Phe Arg Leu Gly Phe Ile Val Glu Lys Ala Gly Glu Asn Phe
 225 230 235 240
 Thr Glu Leu Ala His Lys Gly Gly Val Ile Gly Val Ile Ile Asn Trp
 245 250 255
 Asn Cys Asp Leu Asp Leu Ser Glu Ser Glu Cys Asn Pro Lys Tyr Ser
 260 265 270
 Phe Arg Arg Leu Asp Pro Lys Tyr Asp Pro Ala Ser Ser Gly Tyr Asn
 275 280 285
 Phe Arg Phe Ala Lys Tyr Tyr Lys Ile Asn Gly Thr Thr Thr Arg
 290 295 300
 Thr Leu Ile Lys Ala Tyr Gly Ile Arg Ile Asp Val Ile Val His Gly
 305 310 315 320
 Gln Ala Gly Lys Phe Ser Leu Ile Pro Thr Ile Ile Asn Leu Ala Thr
 325 330 335
 Ala Leu Thr Ser Ile Gly Val Gly Ser Phe Leu Cys Asp Trp Ile Leu
 340 345 350
 Leu Thr Phe Met Asn Lys Asn Lys Leu Tyr Ser His Lys Lys Phe Asp
 355 360 365
 Lys Val Arg Thr Pro Lys His Pro Ser Ser Arg Trp Pro Val Thr Leu
 370 375 380
 Ala Leu Val Leu Gly Gln Ile Pro Pro Pro Pro Ser His Tyr Ser Gln
 385 390 395 400
 Asp Gln Pro Pro Ser Pro Pro Ser Gly Glu Gly Pro Thr Leu Gly Glu
 405 410 415
 Gly Ala Glu Leu Pro Leu Ala Val Gln Ser Pro Arg Pro Cys Ser Ile
 420 425 430
 Ser Ala Leu Thr Glu Gln Val Val Asp Thr Leu Gly Gln His Met Gly
 435 440 445
 Gln Arg Pro Pro Val Pro Glu Pro Ser Gln Gln Asp Ser Thr Ser Thr
 450 455 460
 Asp Pro Lys Gly Leu Ala Gln Leu
 465 470

<210> 5

<211> 1452

<212> DNA

<213> Artificial Sequence

<220>

 <223> Description of Artificial Sequence:/note =
 synthetic construct

<400> 5

gaattcggct gatcccgagg caggtgctag caggagctgg cagcatgggc tcccagggg 60
 ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact 120
 ggcgggtggg cgccctgcag aggctgctgc agtttgggat cgtgggtctat gtggtagggt 180
 gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaacccag ttttccatca 240
 tcaccaaact caaaggggtt tccgtcactc agatcaagga gcttggaac cggctgtggg 300
 atgtggccga ctctgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact 360
 tccttgtgac gccagcccaa gttcagggca gatgccaga gcaccgctec gtccactgg 420

```

ctaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacgggtg 480
taaaaacagg ccagtgtgtg gtgttcaatg ggaccacacag gacctgtgag atctggagtt 540
ggtgcccagt ggagagtggc gttgtgccct cgaggcccct gctggcccag gcccagaact 600
tcacactgtt catcaaaaac acagtcacct tcagcaagtt caacttctct aagtccaatg 660
ccttgagagac ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc 720
cctactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg 780
acctggcggt gctgggtggc tctgtaggca tcagagtcca ctgggattgt gacctggaca 840
ccggggactc tggctgctgg cctcactact ccttccagct gcaggagaag agctacaact 900
tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggccgcacc ctgctcaagc 960
tctatggaat ccgcttcgac atcctcgtca ccgggcaggc agggaaagttc gggctcatcc 1020
ccacggcgtt cacactgggc accggggcag cttggctggg cgtggtcacc tttttctgtg 1080
acctgctact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg 1140
aggccaaggc ccgaaagca accgccaact ctgtgtggag ggagctggcc tttgcatccc 1200
aagcccagct ggccgagtgc ctcagacgga gctcagcacc tgcaccacag gccactgctg 1260
ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa 1320
cccattccgg gagcctgtag ccgtttccct gctggttgag aagagagagg ggctgggcaa 1380
ggaaggaccc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat 1440
caagccgaat tc                                     1452

```

<210> 6

<211> 397

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 6

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Met Asn Cys Ile Ser Asp Phe Phe Thr Tyr Glu Thr Thr Lys Ser Val
1          5          10          15
Val Val Lys Ser Trp Thr Ile Gly Ile Ile Asn Arg Val Val Gln Leu
20          25          30
Leu Ile Ile Ser Tyr Phe Val Gly Trp Val Phe Leu His Glu Lys Ala
35          40          45
Tyr Gln Val Arg Asp Thr Ala Ile Glu Ser Ser Val Val Thr Lys Val
50          55          60
Lys Gly Ser Gly Leu Tyr Ala Asn Arg Val Met Asp Val Ser Asp Tyr
65          70          75          80
Val Thr Pro Pro Gln Gly Thr Ser Val Phe Val Ile Ile Thr Lys Met
85          90          95
Ile Val Thr Glu Asn Gln Met Gln Gly Phe Cys Pro Glu Ser Glu Glu
100         105         110
Lys Tyr Arg Cys Val Ser Asp Ser Gln Cys Gly Pro Glu Pro Leu Pro
115         120         125
Gly Gly Gly Ile Leu Thr Gly Arg Cys Val Asn Tyr Ser Ser Val Leu
130         135         140
Arg Thr Cys Glu Ile Gln Gly Trp Cys Pro Thr Glu Val Asp Thr Val
145         150         155         160
Glu Thr Pro Ile Met Met Glu Ala Glu Asn Phe Thr Ile Phe Ile Lys
165         170         175
Asn Ser Ile Arg Phe Pro Leu Phe Asn Phe Glu Lys Gly Asn Leu Leu
180         185         190
Pro Asn Leu Thr Ala Arg Asp Met Lys Thr Cys Arg Phe His Pro Asp
195         200         205
Lys Asp Pro Phe Cys Pro Ile Leu Arg Val Gly Asp Val Val Lys Phe
210         215         220
Ala Gly Gln Asp Phe Ala Lys Leu Ala Arg Thr Gly Gly Val Leu Gly
225         230         235         240
Ile Lys Ile Gly Trp Val Cys Asp Leu Asp Lys Ala Trp Asp Gln Cys
245         250         255

```

```

Ile Pro Lys Tyr Ser Phe Thr Arg Leu Asp Ser Val Ser Glu Lys Ser
      260      265      270
Ser Val Ser Pro Gly Tyr Asn Phe Arg Phe Ala Lys Tyr Tyr Lys Met
      275      280      285
Glu Asn Gly Ser Glu Tyr Arg Thr Leu Leu Lys Ala Phe Gly Ile Arg
      290      295      300
Phe Asp Val Leu Val Tyr Gly Asn Ala Gly Lys Phe Asn Ile Ile Pro
305      310      315      320
Thr Ile Ile Ser Ser Val Ala Ala Phe Thr Ser Val Gly Val Gly Thr
      325      330      335
Val Leu Cys Asp Ile Ile Leu Leu Asn Phe Leu Lys Gly Ala Asp Gln
      340      345      350
Tyr Lys Ala Lys Lys Phe Glu Glu Val Asn Glu Thr Thr Leu Lys Ile
      355      360      365
Ala Ala Leu Thr Asn Pro Val Tyr Pro Ser Asp Gln Thr Thr Ala Glu
      370      375      380
Lys Gln Ser Thr Asp Ser Gly Ala Phe Ser Ile Gly His
385      390      395

```

<210> 7

<211> 1452

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 7

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gaattcggct gatcccgagg caggtgctag caggagctgg cagcatgggc tccccagggg 60
ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact 120
ggcgggtggg cgccctgcag aggtgctgctc agtttgggat cgtgggtctat gtggtagggg 180
gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaacccag ttttccatca 240
tcacaaaact caaaggggtt tccgtcactc agatcaagga gcttggaac cggtgtggg 300
atgtggccga ctctgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact 360
tccttgtgac gccagcccaa gttcagggca gatgccaga gcacccgtcc gtccactgg 420
ctaaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacgggt 480
taaaaacagg ccagtgtgtg gtgttcaatg ggaccacag gacctgtgag atctggagtt 540
ggtgcccagt ggagagtggc gttgtgccct cgaggcccct gctggcccag gccagaact 600
tcacactgtt catcaaaaac acagtcacct tcagcaagtt caacttctct aagtccaatg 660
ccttgagagc ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc 720
cctactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg 780
acctggcggt gctgggtggc tctgtaggca tcagagtcca ctgggattgt gacctggaca 840
ccggggactc tggctgctgg cctcactact ccttcagct gcaggagaag agctacaact 900
tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgcacc ctgctcaagc 960
tctatggaat ccgcttcgac atcctcgta cgggcaggc agggaaagttc gggctcatcc 1020
ccacggccgt cacactgggc accggggcag cttggctggg cgtggtcacc ttttctgtg 1080
acctgtact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg 1140
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aagcccgact ggccgagtgc ctacagcgga gctcagcacc tgcacccacg gccactgctg 1260
ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa 1320
ccatttcgg gagcctgtag ccgtttccct gctggttgag aagagagagg ggctgggcaa 1380
ggaaggaccc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat 1440
caagccgaat tc

```

1452

<210> 8

<211> 388

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 8

```

Met Ala Gly Cys Cys Ser Ala Leu Ala Ala Phe Leu Phe Glu Tyr Asp
 1              5              10              15
Thr Pro Arg Ile Val Leu Ile Arg Ser Arg Lys Val Gly Leu Met Asn
              20              25              30
Arg Ala Val Gln Leu Leu Ile Leu Ala Tyr Val Ile Gly Trp Val Phe
              35              40              45
Val Trp Glu Lys Gly Tyr Gln Glu Thr Asp Ser Val Val Ser Ser Val
              50              55              60
Thr Thr Lys Val Lys Gly Val Ala Val Thr Asn Thr Ser Lys Leu Gly
65              70              75              80
Phe Arg Ile Trp Asp Val Ala Asp Tyr Val Ile Pro Ala Gln Glu Glu
              85              90              95
Asn Ser Leu Phe Val Met Thr Asn Val Ile Leu Thr Met Asn Gln Thr
              100              105              110
Gln Gly Leu Cys Pro Glu Ile Pro Asp Ala Thr Thr Val Cys Lys Ser
              115              120              125
Asp Ala Ser Cys Thr Ala Gly Ser Ala Gly Thr His Ser Asn Gly Val
              130              135              140
Ser Thr Gly Arg Cys Val Ala Phe Asn Gly Ser Val Lys Thr Cys Glu
145              150              155              160
Val Ala Ala Trp Cys Pro Val Glu Asp Asp Thr His Val Pro Gln Pro
              165              170              175
Ala Phe Leu Lys Ala Ala Glu Asn Phe Thr Leu Leu Val Lys Asn Asn
              180              185              190
Ile Trp Tyr Pro Lys Phe Asn Phe Ser Lys Arg Asn Ile Leu Pro Asn
              195              200              205
Ile Thr Thr Thr Tyr Leu Lys Ser Cys Ile Tyr Asp Ala Lys Thr Asp
              210              215              220
Pro Phe Cys Pro Ile Phe Arg Leu Gly Lys Ile Val Glu Asn Ala Gly
225              230              235              240
His Ser Phe Gln Asp Met Ala Val Glu Gly Gly Ile Met Gly Ile Gln
              245              250              255
Val Asn Trp Asp Cys Asn Leu Asp Arg Ala Ala Ser Leu Cys Leu Pro
              260              265              270
Arg Tyr Ser Phe Arg Arg Leu Asp Thr Arg Asp Val Glu His Asn Val
              275              280              285
Ser Pro Gly Tyr Asn Phe Arg Phe Ala Lys Tyr Tyr Arg Asp Leu Ala
              290              295              300
Gly Asn Glu Gln Arg Thr Leu Ile Lys Ala Tyr Gly Ile Arg Phe Asp
305              310              315              320
Ile Ile Val Phe Gly Lys Ala Gly Lys Phe Asp Ile Ile Pro Thr Met
              325              330              335
Ile Asn Ile Gly Ser Gly Leu Ala Leu Leu Gly Met Ala Thr Val Leu
              340              345              350
Cys Asp Ile Ile Val Leu Tyr Cys Met Lys Lys Arg Leu Tyr Tyr Arg
              355              360              365
Glu Lys Lys Tyr Lys Tyr Val Glu Asp Tyr Glu Gln Gly Leu Ala Ser
              370              375              380
Glu Leu Asp Gln
385

```

<210> 9

<211> 1978

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 9

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ggcagcagggg tccgcaagcc cggctgagag cgcgccatgg ggcagggcggg ctgcaagggg 60
ctctgcctgt cgctgttcga ctacaagacc gagaagtatg tcatcgccaa gaacaagaag 120
gtgggcctgc tgtaccggct gctgcaggcc tccatcctgg cgtacctggg cgtatgggtg 180
ttcctgataa agaagggtta ccaagacgtc gacacctccc tgcagagtgc tgtcatcacc 240
aaagtcaagg gcgtggcctt caccaacacc tcggtatcttg ggcagcggat ctgggatgtc 300
gccgactacg tcattccagc ccagggagag aacgtctttt ttgtgggtcac caacctgatt 360
gtgaccccca accagcggca gaacgtctgt gctgagaatg aaggcattcc tgatggcgcg 420
tgctccaagg acagcgactg ccacgctggg gaagcgggta cagctggaaa cggagtgaag 480
accggccgct gcctgcggag agggaaacttg gccaggggca cctgtgagat ctttgcctgg 540
tgcccgttgg agacaagctc caggccggag gagccattcc tgaaggaggc cgaagacttc 600
accattttca taaagaacca catccgtttc cccaaattca acttctccaa aaacaatgtg 660
atggacgtca aggacagatc ttccctgaaa tcatgccact ttggcccca gaaccactac 720
tgccccatct tccgactggg ctccatcgct cgctgggccc ggagcgactt ccaggatata 780
gccctgcgag gtggcggtgat aggaattaat attgaatgga actgtgatct tgataaagct 840
gcctctgaag gccaccctca ctattctttt agccgtcttg acaataaact ttcaaagtct 900
gtctcctccg ggtacaactt cagatttgcc agatattacc gagacgcagc cggggtggag 960
ttccgcaccc tgatgaaagc ctacgggatc cgctttgacg tgatggtgaa cggcaagggt 1020
gctttcttct gcgacctggg actcatctac ctcatcaaaa agagagagtt ttaccgtgac 1080
aagaagtacg aggaagtga gggcctagaa gacagttccc aggaggccga ggacgaggca 1140
tcggggctgg ggctatctga gcagctcaca tctgggcccag ggctgctggg gatgccggag 1200
cagcaggagc tgcaggagcc acccgaggcg aagcgtggaa gcagcagtca gaaggggaac 1260
ggatctgtgt gccacagct cctggagccc cacaggagca cgtgaattgc ctctgcttac 1320
gttcaggccc tgtcctaacc ccagccgtct agcaccagat gatcccatgc ctttgggaat 1380
cccaggatgc tgcccaacgg gaaatttgta cattgggtgc tatcaatgcc acatcacagg 1440
gaccagccat cacagagcaa agtgacctcc acgtctgatg ctggggtcat caggacggac 1500
ccatcatggc tgtctttttg cccaccccc tgccgtcagt tcttccttcc tccgtggctg 1560
gcttcccga ctagggaacg ggttgtaaat ggggaacatg acttccttcc ggagtccttg 1620
agcacctcag ctaaggaccg cagtgccttg tagagttcct agattacctc actgggaata 1680
gcattgtgct tgtccggaag agggctccat ttggttccag cccactcccc tctgcaagtg 1740
ccacagcttc cctcagagca tactctccag tggatccaag tactctctct cctaaagaca 1800
ccaccttctt gccagctgtt tgcccttagg ccagtacaca gaattaaagt gggggagatg 1860
gcagacgctt tctgggacct gcccaagata tgtattctct gacactctta tttggtcata 1920
aaacaataaa tgggtgcaat ttcaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1978

```

<210> 10

<211> 422

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 10

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Met Gly Gln Ala Gly Cys Lys Gly Leu Cys Leu Ser Leu Phe Asp Tyr
 1           5           10          15
Lys Thr Glu Lys Tyr Val Ile Ala Lys Asn Lys Lys Val Gly Leu Leu
 20          25          30
Tyr Arg Leu Leu Gln Ala Ser Ile Leu Ala Tyr Leu Val Val Trp Val
 35          40          45
Phe Leu Ile Lys Lys Gly Tyr Gln Asp Val Asp Thr Ser Leu Gln Ser
 50          55          60
Ala Val Ile Thr Lys Val Lys Gly Val Ala Phe Thr Asn Thr Ser Asp
 65          70          75          80

```

Leu Gly Gln Arg Ile Trp Asp Val Ala Asp Tyr Val Ile Pro Ala Gln
 85 90 95
 Gly Glu Asn Val Phe Phe Val Val Thr Asn Leu Ile Val Thr Pro Asn
 100 105 110
 Gln Arg Gln Asn Val Cys Ala Glu Asn Glu Gly Ile Pro Asp Gly Ala
 115 120 125
 Cys Ser Lys Asp Ser Asp Cys His Ala Gly Glu Ala Val Thr Ala Gly
 130 135 140
 Asn Gly Val Lys Thr Gly Arg Cys Leu Arg Arg Gly Asn Leu Ala Arg
 145 150 155 160
 Gly Thr Cys Glu Ile Phe Ala Trp Cys Pro Leu Glu Thr Ser Ser Arg
 165 170 175
 Pro Glu Glu Pro Phe Leu Lys Glu Ala Glu Asp Phe Thr Ile Phe Ile
 180 185 190
 Lys Asn His Ile Arg Phe Pro Lys Phe Asn Phe Ser Lys Asn Asn Val
 195 200 205
 Met Asp Val Lys Asp Arg Ser Phe Leu Lys Ser Cys His Phe Gly Pro
 210 215 220
 Lys Asn His Tyr Cys Pro Ile Phe Arg Leu Gly Ser Ile Val Arg Trp
 225 230 235 240
 Ala Gly Ser Asp Phe Gln Asp Ile Ala Leu Arg Gly Gly Val Ile Gly
 245 250 255
 Ile Asn Ile Glu Trp Asn Cys Asp Leu Asp Lys Ala Ala Ser Glu Cys
 260 265 270
 His Pro His Tyr Ser Phe Ser Arg Leu Asp Asn Lys Leu Ser Lys Ser
 275 280 285
 Val Ser Ser Gly Tyr Asn Phe Arg Phe Ala Arg Tyr Tyr Arg Asp Ala
 290 295 300
 Ala Gly Val Glu Phe Arg Thr Leu Met Lys Ala Tyr Gly Ile Arg Phe
 305 310 315 320
 Asp Val Met Val Asn Gly Lys Gly Ala Phe Phe Cys Asp Leu Val Leu
 325 330 335
 Ile Tyr Leu Ile Lys Lys Arg Glu Phe Tyr Arg Asp Lys Lys Tyr Glu
 340 345 350
 Glu Val Arg Gly Leu Glu Asp Ser Ser Gln Glu Ala Glu Asp Glu Ala
 355 360 365
 Ser Gly Leu Gly Leu Ser Glu Gln Leu Thr Ser Gly Pro Gly Leu Leu
 370 375 380
 Gly Met Pro Glu Gln Gln Glu Leu Gln Glu Pro Pro Glu Ala Lys Arg
 385 390 395 400
 Gly Ser Ser Ser Gln Lys Gly Asn Gly Ser Val Cys Pro Gln Leu Leu
 405 410 415
 Glu Pro His Arg Ser Thr
 420

<210> 11

<211> 1452

<212> DNA

<213> Artificial Sequence

<220>

 <223> Description of Artificial Sequence:/note =
 synthetic construct

<400> 11

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gaattcggct gatcccgcg caggtgctag caggagctgg cagcatgggc tccccagggg 60
ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact 120
ggcgggtggg cgccctgcag aggctgctgc agtttgggat cgtggtctat gtggtagggt 180
gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaaccccag ttttccatca 240
tcaccaaact caaaggggtt tccgtcactc agatcaagga gcttggaac cggctgtggg 300

```



```

atgtggccga ctctgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact 360
tccttgtagac gccagcccaa gttcagggca gatgccaga gcaccggtcc gtccactgg 420
ctaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacggtg 480
taaaaacagg ccagtgtgtg gtgttcaatg ggaccacacag gacctgtgag atctggagtt 540
ggtgcccagt ggagagtggc gttgtgccct cgaggcccct gctggcccag gccagaact 600
tcacactgtt catcaaaaac acagtcacct tcagcaagtt caacttctct aagtccaatg 660
ccttgagagac ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc 720
cctactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg 780
acctggcggtt gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca 840
ccggggactc tggctgctgg cctcactact ccttccagct gcaggagaag agctacaact 900
tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgacc ctgctcaagc 960
tctatggaat ccgcttcgac atcctcgtca ccgggcaggc agggaagttc gggctcatcc 1020
ccacggccgt cacactgggc accggggcag cttggctggg cgtggtcacc tttttctgtg 1080
acctgctact gctgtatgtg gatagagaag ccattttcta ctggaggaca aagtatgagg 1140
aggccaaggc cccgaaagca accgccaact ctgtgtggag ggagctggcc tttgcatccc 1200
aagcccgact ggccgagtgc ctcagacgga gctcagcacc tgcaccacag gccactgctg 1260
ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa 1320
ccattccgg gagcctgtag ccgtttccct gctgggtgag aagagagagg ggctgggcaa 1380
ggaaggaccc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat 1440
caagccgaat tc

```

1452

<210> 12

<211> 431

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 12

```

Met Gly Ser Pro Gly Ala Thr Thr Gly Trp Gly Leu Leu Asp Tyr Lys
1          5          10          15
Thr Glu Lys Tyr Val Met Thr Arg Asn Trp Arg Val Gly Ala Leu Gln
20          25          30
Arg Leu Leu Gln Phe Gly Ile Val Val Tyr Val Val Gly Trp Ala Leu
35          40          45
Leu Ala Lys Lys Gly Tyr Gln Glu Arg Asp Leu Glu Pro Gln Phe Ser
50          55          60
Ile Ile Thr Lys Leu Lys Gly Val Ser Val Thr Gln Ile Lys Glu Leu
65          70          75          80
Gly Asn Arg Leu Trp Asp Val Ala Asp Phe Val Lys Pro Pro Gln Gly
85          90          95
Glu Asn Val Phe Phe Leu Val Thr Asn Phe Leu Val Thr Pro Ala Gln
100         105         110
Val Gln Gly Arg Cys Pro Glu His Pro Ser Val Pro Leu Ala Asn Cys
115         120         125
Trp Val Asp Glu Asp Cys Pro Glu Gly Glu Gly Thr His Ser His
130         135         140
Gly Val Lys Thr Gly Gln Cys Val Val Phe Asn Gly Thr His Arg Thr
145         150         155         160
Cys Glu Ile Trp Ser Trp Cys Pro Val Glu Ser Gly Val Val Pro Ser
165         170         175
Arg Pro Leu Leu Ala Gln Ala Gln Asn Phe Thr Leu Phe Ile Lys Asn
180         185         190
Thr Val Thr Phe Ser Lys Phe Asn Phe Ser Lys Ser Asn Ala Leu Glu
195         200         205
Thr Trp Asp Pro Thr Tyr Phe Lys His Cys Arg Tyr Glu Pro Gln Phe
210         215         220
Ser Pro Tyr Cys Pro Val Phe Arg Ile Gly Asp Leu Val Ala Lys Ala
225         230         235         240

```

<400> 13						
aaaacgcagg	gagggaggct	gtcaccatgc	cggcctgctg	cagctgcagt	gatgttttcc	60
agtatgagac	gaacaaagtc	actcggatcc	agagcatgaa	ttatggcacc	attaagtgg	120
tcttccacgt	gatcatcttt	tctcatgttt	gctttgctct	ggtgagtgac	aagctgtacc	180
agcggaaga	gcctgtcttc	agttctgtgc	acaccaaggt	gaaggggata	gcagaggtga	240
aagaggagat	cgtggagaat	ggagtgaaga	cagtgtgtga	cagtgtcttt	gacaccgcag	300
actacacctt	ccctttgcag	gggaactctt	tcttcgtgat	gacaaacttt	gtcaaaacag	360
aaggccaaga	gcagcggttg	tgtcccaggt	atcccacccg	caggacgctc	tgttcctctg	420
accgaggttg	taaaaaggga	tggatggacc	cgcagagcaa	aggaattcag	accggaaggt	480
gtgtagtgca	tgaagggaac	cagaagacct	gtgaagtctc	tgccctggtc	cccatcgagg	540
cagtgggaaga	ggccccccgg	cctgctctct	tgaacagtgc	cgaaaacttc	actgtgtcta	600
tcaagaacaa	tatgcacttc	cccggccaca	actaccacc	gagaacatc	ctggcaggtt	660
taaaccatcac	ttgtaccttc	cacaagactc	agaatccaca	tggtcccat	ttccgactag	720
gagacatctt	ccgagaaaca	ggcgataatt	tttcagatgt	ggcaattcag	ggcggaataa	780
tgggcattga	gatctactgg	gactgcaacc	tagaccgttg	gttccatcac	tgccatcca	840
aatacagttt	ccgtcgcctt	gacgacaaga	ccaccaacgt	gtccttgtac	cctgggtaca	900
acttcagata	cgccaagtat	tacaaggaaa	acaatgttga	gaaacggact	ctgataaaa	960
tcttccggat	cgcttttgac	atcctggttt	tggccaccgg	agggaaaattt	gacattatcc	1020
actgtggtgt	gtacactcgc	tcaaccctct	cctacttcgg	tctggcgcgt	gtgttcacgt	1080
acttcctcat	cgacacttac	tccagtaact	gctgtcgcct	ccatatatt	ccctgggtgca	1140
agtgtgttca	gcctgtgtgt	gtcaacgaat	actactacag	gaagaagtgc	gagtccattg	1200
tggagccaaa	gccgacatta	aagtatgtgt	cctttgtgga	tgaatcccac	attaggatgg	1260
tgaaccagca	gctactaggg	agaagtctgc	aagatgtcaa	gggccaagaa	gtcccaagac	1320
ctgcgatgga	cttcacagat	ttgtccaggc	tgcccttggc	cctccatgac	acacccccga	1380
ttctcggaca	accagaggag	atacagctgc	ttagaaggga	ggcagactcct	agatccagg	1440
atagccccgt	ctggtgccag	tgtggaagct	gcctcccact	tgcactccct	gagagccaca	1500

```

ggcgccctgga ggagctgtgc tgccggaaaa agccggggggc ctgcatcacc acctcagagc 1560
tgttcaggaa gctgggtcctg tccagacacg tcctgcagtt cctcctgctc taccaggagc 1620
ccttgctggc gctggatgtg gattccacca acagccggct gcggcactgt gcctacaggt 1680
gctacgccac ctggcgcttc ggctcccagg acatggctga ctttgccatc ctgcccagct 1740
gctgccgctg gaggatccgg aaagagtttc cgaagagtga agggcagtac agtggcttca 1800
agagtcctta ctgaagccag gcaccgtggc tcacgtctgt aatcccacct ttt 1853

```

<210> 14

<211> 595

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 14

```

Met Pro Ala Cys Cys Ser Cys Ser Asp Val Phe Gln Tyr Glu Thr Asn
1      5      10      15
Lys Val Thr Arg Ile Gln Ser Met Asn Tyr Gly Thr Ile Lys Trp Phe
20     25     30
Phe His Val Ile Ile Phe Ser Tyr Val Cys Phe Ala Leu Val Ser Asp
35     40     45
Lys Leu Tyr Gln Arg Lys Glu Pro Val Ile Ser Ser Val His Thr Lys
50     55     60
Val Lys Gly Ile Ala Glu Val Lys Glu Glu Ile Val Glu Asn Gly Val
65     70     75     80
Lys Lys Leu Val His Ser Val Phe Asp Thr Ala Asp Tyr Thr Phe Pro
85     90     95
Leu Gln Gly Asn Ser Phe Phe Val Met Thr Asn Phe Leu Lys Thr Glu
100    105    110
Gly Gln Glu Gln Arg Leu Cys Pro Glu Tyr Pro Thr Arg Arg Thr Leu
115    120    125
Cys Ser Ser Asp Arg Gly Cys Lys Lys Gly Trp Met Asp Pro Gln Ser
130    135    140
Lys Gly Ile Gln Thr Gly Arg Cys Val Val His Glu Gly Asn Gln Lys
145    150    155    160
Thr Cys Glu Val Ser Ala Trp Cys Pro Ile Glu Ala Val Glu Glu Ala
165    170    175
Pro Arg Pro Ala Leu Leu Asn Ser Ala Glu Asn Phe Thr Val Leu Ile
180    185    190
Lys Asn Asn Ile Asp Phe Pro Gly His Asn Tyr Thr Thr Arg Asn Ile
195    200    205
Leu Pro Gly Leu Asn Ile Thr Cys Thr Phe His Lys Thr Gln Asn Pro
210    215    220
Gln Cys Pro Ile Phe Arg Leu Gly Asp Ile Phe Arg Glu Thr Gly Asp
225    230    235    240
Asn Phe Ser Asp Val Ala Ile Gln Gly Gly Ile Met Gly Ile Glu Ile
245    250    255
Tyr Trp Asp Cys Asn Leu Asp Arg Trp Phe His His Cys His Pro Lys
260    265    270
Tyr Ser Phe Arg Arg Leu Asp Asp Lys Thr Thr Asn Val Ser Leu Tyr
275    280    285
Pro Gly Tyr Asn Phe Arg Tyr Ala Lys Tyr Tyr Lys Glu Asn Asn Val
290    295    300
Glu Lys Arg Thr Leu Ile Lys Val Phe Gly Ile Arg Phe Asp Ile Leu
305    310    315    320
Val Phe Gly Thr Gly Gly Lys Phe Asp Ile Ile Gln Leu Val Val Tyr
325    330    335
Ile Gly Ser Thr Leu Ser Tyr Phe Gly Leu Ala Ala Val Phe Ile Asp
340    345    350

```

Phe Leu Ile Asp Thr Tyr Ser Ser Asn Cys Cys Arg Ser His Ile Tyr
 355 360 365
 Pro Trp Cys Lys Cys Cys Gln Pro Cys Val Val Asn Glu Tyr Tyr Tyr
 370 375 380
 Arg Lys Lys Cys Glu Ser Ile Val Glu Pro Lys Pro Thr Leu Lys Tyr
 385 390 395 400
 Val Ser Phe Val Asp Glu Ser His Ile Arg Met Val Asn Gln Gln Leu
 405 410 415
 Leu Gly Arg Ser Leu Gln Asp Val Lys Gly Gln Glu Val Pro Arg Pro
 420 425 430
 Ala Met Asp Phe Thr Asp Leu Ser Arg Leu Pro Leu Ala Leu His Asp
 435 440 445
 Thr Pro Pro Ile Pro Gly Gln Pro Glu Glu Ile Gln Leu Leu Arg Lys
 450 455 460
 Glu Ala Thr Pro Arg Ser Arg Asp Ser Pro Val Trp Cys Gln Cys Gly
 465 470 475 480
 Ser Cys Leu Pro Ser Gln Leu Pro Glu Ser His Arg Cys Leu Glu Glu
 485 490 495
 Leu Cys Cys Arg Lys Lys Pro Gly Ala Cys Ile Thr Thr Ser Glu Leu
 500 505 510
 Phe Arg Lys Leu Val Leu Ser Arg His Val Leu Gln Phe Leu Leu Leu
 515 520 525
 Tyr Gln Glu Pro Leu Leu Ala Leu Asp Val Asp Ser Thr Asn Ser Arg
 530 535 540
 Leu Arg His Cys Ala Tyr Arg Cys Tyr Ala Thr Trp Arg Phe Gly Ser
 545 550 555 560
 Gln Asp Met Ala Asp Phe Ala Ile Leu Pro Ser Cys Cys Arg Trp Arg
 565 570 575
 Ile Arg Lys Glu Phe Pro Lys Ser Glu Gly Gln Tyr Ser Gly Phe Lys
 580 585 590
 Ser Pro Tyr
 595

<210> 15

<211> 1312

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
 synthetic construct

<400> 15

ggatccagtt cgctgctcc cttccgctcg ctggcttttc cgatgcttgc tgcgcccctg 60
 gccgccgctg cctctcgcgc gcctcctacc cctcggagcc gccgcctaag tgcaggagga 120
 gagaatgacc gaggtgctgt ggccggctgt ccccaacggg acggacgctg ccttcctggc 180
 cggtcggggt tcgtcctggg ggaacagcac ggtcgcctcc actgccgccg tctcctcgtc 240
 gttcaaatgc gccttgacca agacgggctt ccagttttac tacctgccgg ctgtctacat 300
 cttggtattc atcatcggct tcctgggcaa cagcgtggcc atctggatgt tcgtcttcca 360
 catgaagccc tggagcggca tctcgtgta catgttcaat ttggctctgg ccgacttctt 420
 gtacgtgctg actctgccag ccctgatctt ctactacttc aataaaacag actggatctt 480
 cggggatgcc atgtgtaaac tgcagagggt catctttcat gtgaacctct atggcagcat 540
 cttgtttctg acatgcatca gtcccaccg gtacagcggg gtggtgtacc ccctcaagtc 600
 cctggggcgg ctcaaaaaga agaattcgat ctgtatcagc gtgctggtgt ggctcattgt 660
 ggtggtggcg atctccccca tcctcttcta ctcaggtacc ggggtccgca aaaacaaaac 720
 catcacctgt tacgacacca cctcagacga gtacctgcga agttatttca tctacagcat 780
 gtgcaagacc gtggccatgt tctgtgtccc cttggtgctg attctgggct gttacggatt 840
 aattgtgaga gctttgattt acaaagatct ggacaactct cctctgagga gaaaatcgat 900
 ttacctggta atcattgtac tgactgtttt tgctgtgtct tacatccctt tccatgtgat 960

```

gaaaacgatg aacttgaggg cccggttga ttttcagacc ccagcaatgt gtgctttcaa 1020
tgacagggtt tatgccacgt atcaggtgac aagaggtcta gcaagtctca acagttgtgt 1080
ggacccatt ctctatttct tggcgggaga tactttcaga aggagactct cccgagccac 1140
aaggaaagct tctagaagaa gtgaggcaaa tttgcaatcc aagagtgaag acatgaccct 1200
caatatttta cctgagttca agcagaatgg agatacaagc ctgtgaaggc acaagaatct 1260
ccaaacacct ctctgttgta atatggtagg atgcttaaca gaatcaagta ct 1312

```

<210> 16

<211> 373

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 16

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Met Thr Glu Val Leu Trp Pro Ala Val Pro Asn Gly Thr Asp Ala Ala
 1           5           10          15
Phe Leu Ala Gly Pro Gly Ser Ser Trp Gly Asn Ser Thr Val Ala Ser
      20           25           30
Thr Ala Ala Val Ser Ser Ser Phe Lys Cys Ala Leu Thr Lys Thr Gly
      35           40           45
Phe Gln Phe Tyr Tyr Leu Pro Ala Val Tyr Ile Leu Val Phe Ile Ile
      50           55           60
Gly Phe Leu Gly Asn Ser Val Ala Ile Trp Met Phe Val Phe His Met
      65           70           75           80
Lys Pro Trp Ser Gly Ile Ser Val Tyr Met Phe Asn Leu Ala Leu Ala
      85           90           95
Asp Phe Leu Tyr Val Leu Thr Leu Pro Ala Leu Ile Phe Tyr Tyr Phe
      100          105          110
Asn Lys Thr Asp Trp Ile Phe Gly Asp Ala Met Cys Lys Leu Gln Arg
      115          120          125
Phe Ile Phe His Val Asn Leu Tyr Gly Ser Ile Leu Phe Leu Thr Cys
      130          135          140
Ile Ser Ala His Arg Tyr Ser Gly Val Val Tyr Pro Leu Lys Ser Leu
      145          150          155          160
Gly Arg Leu Lys Lys Lys Asn Ala Ile Cys Ile Ser Val Leu Val Trp
      165          170          175
Leu Ile Val Val Val Ala Ile Ser Pro Ile Leu Phe Tyr Ser Gly Thr
      180          185          190
Gly Val Arg Lys Asn Lys Thr Ile Thr Cys Tyr Asp Thr Thr Ser Asp
      195          200          205
Glu Tyr Leu Arg Ser Tyr Phe Ile Tyr Ser Met Cys Thr Thr Val Ala
      210          215          220
Met Phe Cys Val Pro Leu Val Leu Ile Leu Gly Cys Tyr Gly Leu Ile
      225          230          235          240
Val Arg Ala Leu Ile Tyr Lys Asp Leu Asp Asn Ser Pro Leu Arg Arg
      245          250          255
Lys Ser Ile Tyr Leu Val Ile Ile Val Leu Thr Val Phe Ala Val Ser
      260          265          270
Tyr Ile Pro Phe His Val Met Lys Thr Met Asn Leu Arg Ala Arg Leu
      275          280          285
Asp Phe Gln Thr Pro Ala Met Cys Ala Phe Asn Asp Arg Val Tyr Ala
      290          295          300
Thr Tyr Gln Val Thr Arg Gly Leu Ala Ser Leu Asn Ser Cys Val Asp
      305          310          315          320
Pro Ile Leu Tyr Phe Leu Ala Gly Asp Thr Phe Arg Arg Arg Leu Ser
      325          330          335
Arg Ala Thr Arg Lys Ala Ser Arg Arg Ser Glu Ala Asn Leu Gln Ser
      340          345          350

```

Lys Ser Glu Asp Met Thr Leu Asn Ile Leu Pro Glu Phe Lys Gln Asn
 355 360 365
 Gly Asp Thr Ser Leu
 370

<210> 17

<211> 2025

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
 synthetic construct

<400> 17

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cggcacgagg caccocgaga ggagaagcgc agcgcagtgg cgagaggagc cccttggtgc 60
agcagcacta cctgcccaga aaaatgctgg aggctgggcg tggcccagg cctggggacc 120
tgtttttccct gtttcccgca gagttccctg cagcccggtc caggtccagg cgtgtgcatt 180
catgagttag gaacccgtgc aggcgctgag catcctgacc tggagagcag gggctgggtca 240
ggcgatggc agcagacctg gggccctgga atgacaccat caatggcacc tgggatgggg 300
atgagctggg ctacaggtgc cgcttcaacg aggacttcaa gtacgtgctg ctgcctgtgt 360
cctacggcgt ggtgtgctg cttgggctgt gtctgaacgc cgtggcgctc tacatcttct 420
tgtgcccgt caagacctg aatgcgtcca ccacatatat gttccacctg gctgtgtctg 480
atgcactgta tgcggcctcc ctgccgctgc tgggtctatta ctacggcgc gccgaccact 540
ggcccttcag cacggtgctc tgcaagctgg tgcgcttcc cttctacacc aacctttact 600
gcagcatcct cttcctcacc tgcacagcg tgcaccggtg tctgggcgctc ttacgacctc 660
tgcgctccct gcgtggggc cgggcccgt acgctcgccg ggtggcggg gccgtgtggg 720
tggtgtgct gccctgccag gcccctgtgc tctactttgt caccaccagc gcgcgcggg 780
gccgcgtaac ctgccacgac acctcggcac ccgagctctt cagccgcttc gtggcctaca 840
gctcagtcac gctgggcctg ctcttcgagg tgcctttgc cgtcatcctt gtctgttacg 900
tgctcatggc tcggcgactg ctaaagccag cctacgggac ctccggcggc ctccctaggg 960
ccaagcgcaa gtccgtgccc accatcgccg tgggtgctggc tgtcttcgcc ctctgcttcc 1020
tgccattcca cgtcaccgc accctctact actccttccg ctccgtggac ctcagctgcc 1080
acaccctcaa cgccatcaac atggccctaca aggttacccg gccgctggcc agtgctaaca 1140
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ctccgtcatt tgacaggggc tcaggatatt cactctgtgg tccagagtca actgttccca 1560
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agtcacaggc tggccagaaa accctggtaa gtaatgagg ctgagtttgc acagtgtgt 1860
ggaatggact gggtgccacg gtggacttag ctctgaggag tacccccagc ccaagagatg 1920
aacatctggg gactaatatc atagacccat ctggaggctc ccatgggcta ggagcagtgt 1980
gaggctgtaa cttatactaa aggttgtgtt gcctgctaaa aaaaaa 2025

```

<210> 18

<211> 377

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
 synthetic construct

<400> 18

Met Ala Ala Asp Leu Gly Pro Trp Asn Asp Thr Ile Asn Gly Thr Trp
 1 5 10 15
 Asp Gly Asp Glu Leu Gly Tyr Arg Cys Arg Phe Asn Glu Asp Phe Lys
 20 25 30
 Tyr Val Leu Leu Pro Val Ser Tyr Gly Val Val Cys Val Leu Gly Leu
 35 40 45
 Cys Leu Asn Ala Val Ala Leu Tyr Ile Phe Leu Cys Arg Leu Lys Thr
 50 55 60
 Trp Asn Ala Ser Thr Thr Tyr Met Phe His Leu Ala Val Ser Asp Ala
 65 70 75 80
 Leu Tyr Ala Ala Ser Leu Pro Leu Leu Val Tyr Tyr Tyr Ala Arg Gly
 85 90 95
 Asp His Trp Pro Phe Ser Thr Val Leu Cys Lys Leu Val Arg Phe Leu
 100 105 110
 Phe Tyr Thr Asn Leu Tyr Cys Ser Ile Leu Phe Leu Thr Cys Ile Ser
 115 120 125
 Val His Arg Cys Leu Gly Val Leu Arg Pro Leu Arg Ser Leu Arg Trp
 130 135 140
 Gly Arg Ala Arg Tyr Ala Arg Arg Val Ala Gly Ala Val Trp Val Leu
 145 150 155 160
 Val Leu Ala Cys Gln Ala Pro Val Leu Tyr Phe Val Thr Thr Ser Ala
 165 170 175
 Arg Gly Gly Arg Val Thr Cys His Asp Thr Ser Ala Pro Glu Leu Phe
 180 185 190
 Ser Arg Phe Val Ala Tyr Ser Ser Val Met Leu Gly Leu Leu Phe Ala
 195 200 205
 Val Pro Phe Ala Val Ile Leu Val Cys Tyr Val Leu Met Ala Arg Arg
 210 215 220
 Leu Leu Lys Pro Ala Tyr Gly Thr Ser Gly Gly Leu Pro Arg Ala Lys
 225 230 235 240
 Arg Lys Ser Val Arg Thr Ile Ala Val Val Leu Ala Val Phe Ala Leu
 245 250 255
 Cys Phe Leu Pro Phe His Val Thr Arg Thr Leu Tyr Tyr Ser Phe Arg
 260 265 270
 Ser Leu Asp Leu Ser Cys His Thr Leu Asn Ala Ile Asn Met Ala Tyr
 275 280 285
 Lys Val Thr Arg Pro Leu Ala Ser Ala Asn Ser Cys Leu Asp Pro Val
 290 295 300
 Leu Tyr Phe Leu Ala Gly Gln Arg Leu Val Arg Phe Ala Arg Asp Ala
 305 310 315 320
 Lys Pro Pro Thr Gly Pro Ser Pro Ala Thr Pro Ala Arg Arg Arg Leu
 325 330 335
 Gly Leu Arg Arg Ser Asp Arg Thr Asp Met Gln Arg Ile Gly Asp Val
 340 345 350
 Leu Gly Ser Ser Glu Asp Phe Arg Arg Thr Glu Ser Thr Pro Ala Gly
 355 360 365
 Ser Glu Asn Thr Lys Asp Ile Arg Leu
 370 375

<210> 19

<211> 1163

<212> DNA

<213> Artificial Sequence

<220>

 <223> Description of Artificial Sequence:/note =
 synthetic construct

<400> 19

ggcgccttcac ccagtaaaga gggaccatga gcatggccaa cttcacgggg gggaggaact 60

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```

cggtgcacctt ccatgaggaa ttcaagcagg tcctgctgcc cctgggtctac tcagtgggtg 120
tcctactggg gctgccactc aatgccgttg tcattgggca gatctggctg gcccgcaagg 180
cggttgaccg caccaccatc tacatgctga acctggccat ggccgacctg ctttatgtct 240
gctccctccc tctcctcatc tacaactaca cccagaagga ttactggccc tttggggact 300
tcacctgcaa attcgtccgc ttccagttct acaccaacct gcacggcagc atcctcttcc 360
tcacctgcat cagcgtccag cgctacatgg ggatctgcca ccccttgccc tcgtggcaca 420
aaaagaaggg aaagaagctg acgtggctgg tgtgtgctgc cgtgtgggtc atcgtcatcg 480
cccagtgccct gccacacctt gtcttcgcct ccaccggcac gcagaggaat cgcactgtct 540
gctatgacct gagcccccg gaccgctcca catcctactt cccctatggc atcacgttga 600
ccatcactgg cttcctgctg cccttcgcag ccctcctggc ctgctactgc agcatggccc 660
gcatcctgtg ccagaaagac gagctgattg gcttggcggt gcacaagaag aaggacaagg 720
ccgtgcgcat gatcatcatc gttgtcatcg tcttctccat cagcttcttc ccttccacc 780
tcaccaagac catctacctg atcgtccgct cctcagccag cttgccctgc cctaccctgc 840
aggcttttgc cattgcctac aagtgcacgc ggccctttgc cagcatgaac agcgtcctcg 900
accccatcct cttctacttc acccagcgca agtttctgta gagcaccgcg tatctcctgg 960
acaagatgag ctccaagtgg cggcaagacc actgcatcag ctacggctcc taggtggacg 1020
aggccacctc ggtgtcaccg gggctgggca tggagcaatt tgggttgaag ctgcatgggtg 1080
cggagatggg gatgagccca gagtgcctgc ggtgccccat ctctggaggt gttggagatt 1140
agattggatg gggctctggg ccc 1163

```

<210> 20

<211> 328

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 20

```

Met Ser Met Ala Asn Phe Thr Gly Gly Arg Asn Ser Cys Thr Phe His
1      5      10      15
Glu Glu Phe Lys Gln Val Leu Leu Pro Leu Val Tyr Ser Val Val Phe
20     25     30
Leu Leu Gly Leu Pro Leu Asn Ala Val Val Ile Gly Gln Ile Trp Leu
35     40     45
Ala Arg Lys Ala Leu Thr Arg Thr Thr Ile Tyr Met Leu Asn Leu Ala
50     55     60
Met Ala Asp Leu Leu Tyr Val Cys Ser Leu Pro Leu Leu Ile Tyr Asn
65     70     75     80
Tyr Thr Gln Lys Asp Tyr Trp Pro Phe Gly Asp Phe Thr Cys Lys Phe
85     90     95
Val Arg Phe Gln Phe Tyr Thr Asn Leu His Gly Ser Ile Leu Phe Leu
100    105    110
Thr Cys Ile Ser Val Gln Arg Tyr Met Gly Ile Cys His Pro Leu Ala
115    120    125
Ser Trp His Lys Lys Lys Gly Lys Lys Leu Thr Trp Leu Val Cys Ala
130    135    140
Ala Val Trp Phe Ile Val Ile Ala Gln Cys Leu Pro Thr Phe Val Phe
145    150    155    160
Ala Ser Thr Gly Thr Gln Arg Asn Arg Thr Val Cys Tyr Asp Leu Ser
165    170    175
Pro Pro Asp Arg Ser Thr Ser Tyr Phe Pro Tyr Gly Ile Thr Leu Thr
180    185    190
Ile Thr Gly Phe Leu Leu Pro Phe Ala Ala Ile Leu Ala Cys Tyr Cys
195    200    205
Ser Met Ala Arg Ile Leu Cys Gln Lys Asp Glu Leu Ile Gly Leu Ala
210    215    220
Val His Lys Lys Lys Asp Lys Ala Val Arg Met Ile Ile Ile Val Val
225    230    235    240

```



```

Ile Val Phe Ser Ile Ser Phe Phe Pro Phe His Leu Thr Lys Thr Ile
      245                      250                      255
Tyr Leu Ile Val Arg Ser Ser Ala Ser Leu Pro Cys Pro Thr Leu Gln
      260                      265                      270
Ala Phe Ala Ile Ala Tyr Lys Cys Thr Arg Pro Phe Ala Ser Met Asn
      275                      280                      285
Ser Val Leu Asp Pro Ile Leu Phe Tyr Phe Thr Gln Arg Lys Phe Arg
      290                      295                      300
Glu Ser Thr Arg Tyr Leu Leu Asp Lys Met Ser Ser Lys Trp Arg Gln
      305                      310                      315                      320
Asp His Cys Ile Ser Tyr Gly Ser
      325

```

<210> 21

<211> 1429

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 21

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aagggagctt gggtaggggc caggctagcc tgagtgcacc cagatgcgct tctgtcagct 60
ctccctagtg cttcaaccac tgctctccct gctctacttt ttttgctcca gctcaggat 120
gggggtgggc agggaaatcc tgccaccctc acttctcccc tccccatctc cagggggggc 180
atggccagta cagagtcctc cctgttgaga tccctaggcc tcagcccagg tcctggcagc 240
agtgaagtg agctggactg ttggtttgat gaggatttca agttcatcct gctgcctgtg 300
agctatgcag ttgtctttgt gctgggcttg ggccttaacg ccccaaccct atggctcttc 360
atcttcgcgc tccgaccctg ggatgcaacg gccacctaca tgttccacct ggcattgtca 420
gacaccttgt atgtgctgtc gctgcccacc ctcatctact attatgcagc ccacaaccac 480
tgcccttttg gcactgagat ctgcaagtcc gtccgctttc ttttctattg gaacctctac 540
tgcaagtgtc ttttcctcac ctgcatcagc gtgcaccgct acctgggcat ctgccacca 600
cttcgggcac tacgtggggc ccgccctcgc ctgcaggcc ttctctgcct ggcagtttgg 660
ttggtcgtag ccggtgcctc cgtgcccac ctgttctttg tcacaaccag caacaaagg 720
accaccgtcc tgtgccaatga caccactcgg cctgaagagt ttgaccacta tgtgcacttc 780
agctcggcgg tcatggggct gctctttggc gtgccctgcc tggtcactct tgtttgctat 840
ggactcatgg ctgcgcct gtatcagccc ttgccaggct ctgcacagtc gtcttctcgc 900
ctccgctctc tccgcaccat agctgtggtg ctgactgtct ttgctgtctg cttcgtgcct 960
ttccacatca cccgcaccat ttactacctg gccaggctgt tggaaagtga ctgccagta 1020
ctgaacattg tcaacgtggt ctataaagt actcgcccc tggccagtgc caacagctgc 1080
ctggatcctg tgctctactt gctcactggg gacaaatctc gacgtcagct ccgtcagctc 1140
tgtggtggtg gcaagcccca gccccgcacg gctgcctctt ccctggcact agtgtccctg 1200
cctgaggata gcagctgcag gtgggcgggc acccccagc acagtagctg ctctactcct 1260
agggcagata gattgtaaca cgggaagccg ggaagtgaga gaaaagggga tgagtgcagg 1320
gcagaggtga gggaacccaa tagtgatacc tggtgaagtg cttcttctc ttttccaggc 1380
tctggagaga agccctcacc ctgagggttg ccaggagggc agggatatc 1429

```

<210> 22

<211> 365

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 22

```

Met Ala Ser Thr Glu Ser Ser Leu Leu Arg Ser Leu Gly Leu Ser Pro
  1                      5                      10                      15

```

Gly Pro Gly Ser Ser Glu Val Glu Leu Asp Cys Trp Phe Asp Glu Asp
 20 25 30
 Phe Lys Phe Ile Leu Leu Pro Val Ser Tyr Ala Val Val Phe Val Leu
 35 40 45
 Gly Leu Gly Leu Asn Ala Pro Thr Leu Trp Leu Phe Ile Phe Arg Leu
 50 55 60
 Arg Pro Trp Asp Ala Thr Ala Thr Tyr Met Phe His Leu Ala Leu Ser
 65 70 75 80
 Asp Thr Leu Tyr Val Leu Ser Leu Pro Thr Leu Ile Tyr Tyr Tyr Ala
 85 90 95
 Ala His Asn His Trp Pro Phe Gly Thr Glu Ile Cys Lys Phe Val Arg
 100 105 110
 Phe Leu Phe Tyr Trp Asn Leu Tyr Cys Ser Val Leu Phe Leu Thr Cys
 115 120 125
 Ile Ser Val His Arg Tyr Leu Gly Ile Cys His Pro Leu Arg Ala Leu
 130 135 140
 Arg Trp Gly Arg Pro Arg Leu Ala Gly Leu Leu Cys Leu Ala Val Trp
 145 150 155 160
 Leu Val Val Ala Gly Cys Leu Val Pro Asn Leu Phe Phe Val Thr Thr
 165 170 175
 Ser Asn Lys Gly Thr Thr Val Leu Cys His Asp Thr Thr Arg Pro Glu
 180 185 190
 Glu Phe Asp His Tyr Val His Phe Ser Ser Ala Val Met Gly Leu Leu
 195 200 205
 Phe Gly Val Pro Cys Leu Val Thr Leu Val Cys Tyr Gly Leu Met Ala
 210 215 220
 Arg Arg Leu Tyr Gln Pro Leu Pro Gly Ser Ala Gln Ser Ser Ser Arg
 225 230 235 240
 Leu Arg Ser Leu Arg Thr Ile Ala Val Val Leu Thr Val Phe Ala Val
 245 250 255
 Cys Phe Val Pro Phe His Ile Thr Arg Thr Ile Tyr Tyr Leu Ala Arg
 260 265 270
 Leu Leu Glu Ala Asp Cys Arg Val Leu Asn Ile Val Asn Val Val Tyr
 275 280 285
 Lys Val Thr Arg Pro Leu Ala Ser Ala Asn Ser Cys Leu Asp Pro Val
 290 295 300
 Leu Tyr Leu Leu Thr Gly Asp Lys Tyr Arg Arg Gln Leu Arg Gln Leu
 305 310 315 320
 Cys Gly Gly Gly Lys Pro Gln Pro Arg Thr Ala Ala Ser Ser Leu Ala
 325 330 335
 Leu Val Ser Leu Pro Glu Asp Ser Ser Cys Arg Trp Ala Ala Thr Pro
 340 345 350
 Gln Asp Ser Ser Cys Ser Thr Pro Arg Ala Asp Arg Leu
 355 360 365

<210> 23

<211> 1571

<212> DNA

<213> Artificial Sequence

<220>

 <223> Description of Artificial Sequence:/note =
 synthetic construct

<400> 23

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 caggcacaga actgactggc agcaggggct gctccacgag tgggaatttg ctccagcact 120
 tcacggactg caagcgaggc acttgctaac tcttgataa caagacctct gccagaagaa 180
 ccatggcttt ggaaggcgga gttaggctg aggagatggg tgcggctctc agtgagcccc 240
 tgctccctg aacataggaa acccacctgg gcagccatgg aatgggacaa tggcacaggc 300

```

caggctctgg gcttgccacc caccacctgt gtctaccgcg agaacttcaa gcaactgctg 360
ctgccacctg tgtattcggc ggtgctggcg gctggcctgc cgctgaacat ctgtgtcatt 420
accagatct gcacgtcccg ccgggcccctg acccgcacgg ccgtgtacac cctaaacctt 480
gctctggctg acctgctata tgctgctcc ctgcccctgc tcatctacaa ctatgcccac 540
ggtgatcact ggcccttttg cgacttcgcc tgccgcctgg tccgcttcct cttctatgcc 600
aacctgcacg gcagcatcct ctccctcacc tgcatacagc tccagcgcta cctgggcatc 660
tgccaccgcg tggccccctg gcacaaacgt gggggccgccc gggctgcctg gctagtgtgt 720
gtagccgtgt ggctggccgt gacaaccagc tgccctgccc cagccatctt cgctgccaca 780
ggcatccagc gtaaccgcac tgtctgctat gacctcagcc cgctgccctt ggccaccac 840
tatatgcctt atggcatggc tctcactgtc atcggttcc tgctgccctt tgctgccctg 900
ctggcctgct actgtctcct ggctgcccgc ctgtgcccgc aggatggccc ggcagagcct 960
gtggcccagg agcggcgtgg caaggcggcc cgcatggccc tgggtggggc tgctgccttt 1020
gccatcagct tcctgccttt tcacatcacc aagacagcct acctggcagt gcgctcgacg 1080
ccgggcgtcc cctgcactgt attggaggcc tttgcagcgg cctacaaagg cagcggccg 1140
tttgccagtg ccaacagcgt gctggacccc atcctcttct acttcaccca gaagaagttc 1200
cgccggcgac cacatgagct cctacagaaa ctcacagcca aatggcagag gcagggtcgc 1260
tgagtcctcc aggtcctggg cagccttcct atttgccatt gtgtccgggg caccaggagc 1320
cccaccaacc ccaaaccatg cggagaatta gagttcagct cagctgggca tggagttaag 1380
atccctcaca ggaccagaa gctcaccaaa aactatttct tcagccctt ctctggccca 1440
gaccctgtgg gcatggagat ggacagacct gggcctggct cttgagaggt ccagtcagc 1500
catggagagc tggggaaacc acattaaggt gctcacaaaa atacagtgtg acgtgtactg 1560
tcaaaaaaaaa a 1571

```

<210> 24

<211> 328

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 24

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Met Glu Trp Asp Asn Gly Thr Gly Gln Ala Leu Gly Leu Pro Pro Thr
 1           5           10          15
Thr Cys Val Tyr Arg Glu Asn Phe Lys Gln Leu Leu Leu Pro Pro Val
 20          25          30
Tyr Ser Ala Val Leu Ala Ala Gly Leu Pro Leu Asn Ile Cys Val Ile
 35          40          45
Thr Gln Ile Cys Thr Ser Arg Arg Ala Leu Thr Arg Thr Ala Val Tyr
 50          55          60
Thr Leu Asn Leu Ala Leu Ala Asp Leu Leu Tyr Ala Cys Ser Leu Pro
 65          70          75          80
Leu Leu Ile Tyr Asn Tyr Ala Gln Gly Asp His Trp Pro Phe Gly Asp
 85          90          95
Phe Ala Cys Arg Leu Val Arg Phe Leu Phe Tyr Ala Asn Leu His Gly
100          105          110
Ser Ile Leu Phe Leu Thr Cys Ile Ser Phe Gln Arg Tyr Leu Gly Ile
115          120          125
Cys His Pro Leu Ala Pro Trp His Lys Arg Gly Gly Arg Arg Ala Ala
130          135          140
Trp Leu Val Cys Val Ala Val Trp Leu Ala Val Thr Thr Gln Cys Leu
145          150          155          160
Pro Thr Ala Ile Phe Ala Ala Thr Gly Ile Gln Arg Asn Arg Thr Val
165          170          175
Cys Tyr Asp Leu Ser Pro Pro Ala Leu Ala Thr His Tyr Met Pro Tyr
180          185          190
Gly Met Ala Leu Thr Val Ile Gly Phe Leu Leu Pro Phe Ala Ala Leu
195          200          205
Leu Ala Cys Tyr Cys Leu Leu Ala Cys Arg Leu Cys Arg Gln Asp Gly
210          215          220

```

```

Pro Ala Glu Pro Val Ala Gln Glu Arg Arg Gly Lys Ala Ala Arg Met
225                230                235                240
Ala Val Val Val Ala Ala Phe Ala Ile Ser Phe Leu Pro Phe His
                245                250                255
Ile Thr Lys Thr Ala Tyr Leu Ala Val Arg Ser Thr Pro Gly Val Pro
                260                265                270
Cys Thr Val Leu Glu Ala Phe Ala Ala Tyr Lys Gly Thr Arg Pro
                275                280                285
Phe Ala Ser Ala Asn Ser Val Leu Asp Pro Ile Leu Phe Tyr Phe Thr
                290                295                300
Gln Lys Lys Phe Arg Arg Pro His Glu Leu Leu Gln Lys Leu Thr
305                310                315                320
Ala Lys Trp Gln Arg Gln Gly Arg
                325

```

<210> 25

<211> 1116

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 25

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gccagcaatg gcctggccct gtaccgcttc agcatccgga agcagcgccc atggcacccc 180
gcegtggtct tctctgtcca gctggcagtc agcgacctgc tctgcgtctt gacgctgccc 240
ccgctggccg cctacctcta tcccccaag cactggcgct atggggaggg cgcgtgccgc 300
ctggagcgct tcctctttac ctgcaacctg ctgggcagcg tcattctcat cacctgcac 360
agcctcaacc gctacctggg catcgtgcac cccttcttcg cccgaagcca cctgcgaccc 420
aagcacgctt gggccgtgag cgctgccggc tgggtcctgg ccgccctgct ggccatgccc 480
acactcagct tctccacct gaagaggccg cagcaggggg cgggcaactg cagcgtggcc 540
aggcccagg cctgcatcaa gtgtctgggg acagcagacc acgggctggc ggcctacaga 600
gcgtatagcc tgggtctggc ggggttgggc tgcggcctgc cgctgctgct cacgctggca 660
gcctacggcg cctcggggcg ggcgctgcta cgcagcccag gcatgactgt ggcggagaag 720
ctgcgtgtgg cagcgttggg ggccagtggg gtggccctct acgccagctc ctatgtgccc 780
taccacatca tgcgggtgct caacgtggat gctcggcggc gctggagcac ccgctgccc 840
agctttgcag acatagccca ggccacagca gccctggagc tggggcccta cgtgggctac 900
caggtgatgc ggggcctcat gccctggcc ttctgtgtcc accctctact ctacatggcc 960
gcagtgccca gcctgggctg ctgctgccga cactgccccg gctacaggga cagctggaac 1020
ccagaggacg ccaagagcac tggccaagcc ctgccccca atgccacagc cgccccctaa 1080
ccgtcagagc cccagtcccc tgagctgagc caatga 1116

```

<210> 26

<211> 371

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 26

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Met Asp Arg Gly Ala Lys Ser Cys Pro Ala Asn Phe Leu Ala Ala Ala
1                5                10                15
Asp Asp Lys Leu Ser Gly Phe Gln Gly Asp Phe Leu Trp Pro Ile Leu
                20                25                30

```

Val Val Glu Phe Leu Val Ala Val Ala Ser Asn Gly Leu Ala Leu Tyr
 35 40 45
 Arg Phe Ser Ile Arg Lys Gln Arg Pro Trp His Pro Ala Val Val Phe
 50 55 60
 Ser Val Gln Leu Ala Val Ser Asp Leu Leu Cys Ala Leu Thr Leu Pro
 65 70 75 80
 Pro Leu Ala Ala Tyr Leu Tyr Pro Pro Lys His Trp Arg Tyr Gly Glu
 85 90 95
 Ala Ala Cys Arg Leu Glu Arg Phe Leu Phe Thr Cys Asn Leu Leu Gly
 100 105 110
 Ser Val Ile Phe Ile Thr Cys Ile Ser Leu Asn Arg Tyr Leu Gly Ile
 115 120 125
 Val His Pro Phe Phe Ala Arg Ser His Leu Arg Pro Lys His Ala Trp
 130 135 140
 Ala Val Ser Ala Ala Gly Trp Val Leu Ala Ala Leu Leu Ala Met Pro
 145 150 155 160
 Thr Leu Ser Phe Ser His Leu Lys Arg Pro Gln Gln Gly Ala Gly Asn
 165 170 175
 Cys Ser Val Ala Arg Pro Glu Ala Cys Ile Lys Cys Leu Gly Thr Ala
 180 185 190
 Asp His Gly Leu Ala Ala Tyr Arg Ala Tyr Ser Leu Val Leu Ala Gly
 195 200 205
 Leu Gly Cys Gly Leu Pro Leu Leu Thr Leu Ala Ala Tyr Gly Ala
 210 215 220
 Leu Gly Arg Ala Val Leu Arg Ser Pro Gly Met Thr Val Ala Glu Lys
 225 230 235 240
 Leu Arg Val Ala Ala Leu Val Ala Ser Gly Val Ala Leu Tyr Ala Ser
 245 250 255
 Ser Tyr Val Pro Tyr His Ile Met Arg Val Leu Asn Val Asp Ala Arg
 260 265 270
 Arg Arg Trp Ser Thr Arg Cys Pro Ser Phe Ala Asp Ile Ala Gln Ala
 275 280 285
 Thr Ala Ala Leu Glu Leu Gly Pro Tyr Val Gly Tyr Gln Val Met Arg
 290 295 300
 Gly Leu Met Pro Leu Ala Phe Cys Val His Pro Leu Leu Tyr Met Ala
 305 310 315 320
 Ala Val Pro Ser Leu Gly Cys Cys Cys Arg His Cys Pro Gly Tyr Arg
 325 330 335
 Asp Ser Trp Asn Pro Glu Asp Ala Lys Ser Thr Gly Gln Ala Leu Pro
 340 345 350
 Leu Asn Ala Thr Ala Ala Pro Lys Pro Ser Glu Pro Gln Ser Arg Glu
 355 360 365
 Leu Ser Gln
 370

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A01N 43/04; A61K 31/70

US CL : 514/42, 43, 44, 45, 46, 47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST, CAPLUS, USPATFULL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,063,596 A (LAL et al.) 16 May 2000 (16.05.2000).	1-66
A	US 2001/0025099 A1 (ELSHOURBAGY et al.) 27 September 2001 (27.09.2001).	25-50

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Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Patrick T. Lewis Telephone No. 703-308-0196

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